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ELECTROMAGNETIC MODIFICATION OF DISUSE OSTEOPOROSIS

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INTRODUCTION: LONG RANGE OBJECTIVES

The length of manned space flight, currently, is limited by the sustained effects of weightlessness on the skeleton. Studies of astronauts and cosmonauts, thus far, indicate progressive loss of skeletal mass in space, despite a variety of countermeasures. If manned orbital flight, in excess of four months, or prolonged missions, such as exploration of Mars, are to be undertaken, new methods are required to maintain bone mass in the face of longterm 0-G environment. During this contract period it was established that low intensity, time-varying electromagnetic fields with highly specific pulse characteristics can prevent localized disuse osteoporosis in the rat. All indications, at the present time, point to the likelihood that this methodology can be adapted, practically, for the manned space program and will prove to be an effective means of combatting the bone loss normally resulting from weightlessness.

The importance of the program for the national effort in space, however, is matched fully by a probable "spin'off" for a national health care problem of major medical and economic significance, namely, post-menopausal and senile osteoporosis. These two (or one) disorders affect at least 5,000,000 females above the age of 65 years in the United States alone. Therapeutic measures aimed at arresting or improving osteoporosis have centered, mainly, on nutritional and hormonal factors, while equally important electromechanical factors have been largely ignored. It seems quite clear, even at this early stage that improvements and adaptations of technology, ultimately, may provide an effective therapy for this condition.

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OBJECTIVE: Conduct Pilot Studies to Develop a Rat Model Producing:

- sufficient degree of osteoporosis
- in a short enough interval (trying to establish a 28-day model)
- with sufficient reproducibility

This model was designed to be used to study possible electromagnetic modification of disuse osteoporosis

APPROACH to
EVOLUTION OF THE MODEL

A. Survey of the literature

B. 4 models were chosen, initially, for study: (By May, 1976 these pilot studies had been completed)

1. Section of the tendo Achillis to produce disuse osteoporosis in the os calci.

2. Section of the sciatic nerve for the same purpose.

3. Section of tendons and nerves of the rat tail to produce osteoporosis of the vertebral bodies of that structure.

4. Subcutaneous pocket in the flank of the rat for one hind limb (one limb skinned and inserted into pocket).

EXPT. For each pilot study above a group of 10-20 rats (Strain B as described later) were used. Sacrifice was set for 30, 45 and 60 days postoperatively. Results were evaluated using the following techniques: radiographic, histologic and mechanical testing (compression testing of whole bone using the Instron).

RESULTS: None of the 4 models, in our hands, met the model criteria sufficiently:

Pilot Model #1 gave only a small % change from control values.

Pilot Model #2 seemed to produce more uniform results, although the rate of bone loss did not permit us the latitude we had hoped for in perfecting a 28-day model.

Pilot model #3 demonstrated variable amounts of returning function during the study periods. Osteoporosis of vertebral bodies in these animals is small in amount and unpredictable in its occurrence. On these bases, we have discarded this approach.

Pilot Model #4 was able to produce only minor loss. In addition, this model produces only one extremity for analyses.

It was evident from pilot studies conducted that casts applied solely to the hind limbs were not practical.

C. Development of body spica cast immobilization to enhance results of pilot models.

A visit to Dr. Emily Holton at NASA-Ames (April '76) and observing her method of hind limb suspension to produce bone loss gave the idea that plaster may be substituted for this purpose.

EXPT. To study the tolerance of mature rats to total body spica immobilization (May - June '76): 25 rats.

RESULTS: It was found that it could be made practical and would result in significant and reproducible bone loss in femurs, tibiae and os calci within 45 days.

D. The "Combined Effects" Model was first studied in June '76.

EXPT. The final model: combined effects of sciatic nerve section, removal of the gastrocnemius, soleus and tendo Achillis <u>and</u> application of a total body spica. More than 60 rats surveyed between June and October '76.

RESULTS: This model produced significant degree of bone loss in the os calci and in the proximal and distal tibiae within 28 days after operation: rapid and profound, localized hind limb osteoporosis. (SEE Figure 1 R)

E. Casting problems with rat total spica

- Casting pressure problems: in the first pilot studies using the coils in during the summer of '76 it was noted that the rats suffered from skin infection of the lower extremities (in 8 of the 10 animals). Perfecting of casting techniques to avoid this problem was emphasized.

- In one series of experiments ("P" Series) the hind limbs of the animals are left uncasted. This approach is considered to cut down on periostitis and mortality problems.

- Since it was noted that disuse robbed the animals of body weight, two translucent Kirschner wires were placed through two proximal tail vertebral bodies to prevent the rat s from wiggling free from the spica.

OBJECTIVE: To determine the effectiveness of pulsing electromagnetic fields (PEMF), produced by the single repetitive pulse or the repetitive pulse burst signal, in preventing bone loss associated with disuse in the "combined approach" rat osteoporosis model.

EXPERIMENTAL DESIGN

all groups fed ad libitum and sacrificed after 28 days

all groups fed ad libitum and sacrificed after 28 days	Group I	Standard Laboratory Cage Controls: Unoperated/ Uncasted. (This group couldn't be operated on because this has proven unpractical due to the animal's ulceration and chewing of insensate feet.) Dimensions of the cages: 23 cm x 45 cm.
	Group II	Operated and Casted Controls (without coils)
	Group III	Experimental Group: Operated, casted and exposed to pulsing electromagnetic fields (employing two main pulse patterns: either single pulse or pulse burst).

EXPERIMENTAL ANIMALS

Sprague Dawley Rats, adult, female, non-breeding 260± 10 gram were used in these studies.

Two strains of these animals were employed during this contract period.

1. Strain A Rats were raised in a pathogen free environment. These animals are characterized by a normal weight gain of 1-2 grams/week. (In earlier reports these rats were referred to as inbred rats.)

2. Strain B Rats were raised in a normal environment and gained as much as 8 - 10 grams/week. These animals are bred at Royal Hart Breeders. (In earlier reports these rats were referred to as non-inbreds.)

Both Strains of rats were supplied to these laboratories by Camm Labs.

These studies started with Strain B Rats and switched to Strain A Rats in January '78 to minimize variability in weight gain in the animals.

Group II and Group III Rat Model: <u>Final "combined approach" disuse osteoporosis model in the rat</u>.

- As described in the previous section of this report, the rats in these two groups had both hind limbs immobilized in a <u>plaster spica,</u> from upper thorax to the toes. (In the "P" Series the hind limbs were uncasted.) The animals were further fixed by two transcutaneous Kirschner wires piercing two proximal tail vertebral bodies. These pins were, in turn, placed in two adjustable "C" shaped plastic yokes which were coupled to a lucite bar extending caudally along the dorsum of the cast. A plaster pedestal attached to the ventral surface permitted the animal to be "suspended" in space and freed the forequarters for feeding. (SEE Figure II R)

- The <u>operative procedure</u>: the bilateral removal of the gastroenemius and soleus muscles, together with the tendo Achilles, and a neurectomy of the posterior tibial and peroneal nerves at the level of the popiteal space. Following skin closure the animals were casted as noted above.

See Figure III R.

Experimental Group III: After operating and casting the experimental group was exposed for 24 hours per day to pulsing fields generated by Helmholtz-aiding coils which flanked both lower extremities so they were within the coil ports or "windows". (In later studies larger coils were used.) See Figure 4R.

Two main types of highly specific electromagnetic pulses* were employed in these studies (and also those with mice and rabbits):

1. Single, quasi-rectangular pulse, lasting 300 μsec, repeating at 70 Hz, and inducing 1 - 1.5 mv/cm of bone.

    Original criteria for chosing this pulse pattern: Pulses with these characteristics had been shown to affect bone repair in animals and humans and to cause significant amts. of calcium release by cells and 7-day old chick embryonic limb rudiments in culture. Exposure to pulses of these characteristics have no known deleterious effects.

2. The second pulse pattern is characterized by a "train" of quasi-rectangular pulses, lasting 200 μsec, repeating at 5 - 15 Hz, and inducing 1 - 1.5 mv/cm bone, as above. These are designed to group a series of ≈20, 200 μsec wide pulses less than 50 μsec apart, in a burst lasting 5 msec and recurring at a repetition rate of 5 to 15 Hz. (This pulse pattern is referred to as the pulse train or repetitive pulse burst in this discussion.)

    Original criteria for chosing this pulse parameter: As a result of programs in pseudarthroses in humans, it became evident in mid-1976 that more rapid healing occurred if a "pulse train" was used, rather than a single pulse. In other phases of the laboratories' programs this pulse pattern was shown to cause increased protein synthesis in vitro and not to affect calcium release in culture. Exposure to pulses of these characteristics have no known deleterious effects.

Electromagnetic equipment monitoring:

The equipment is calibrated before it leaves Electro-Biology Inc. and

* These studies were accomplished with PEMF circuitry and coils leased from Electro-Biology Inc. (W. Caldwell, New Jersey). No modification of equipment or waveform characteristics were required or engendered by this program.

arrives at these laboratories with signal specification sheets. These specifications are checked before starting the experiment and at periodic intervals. This is done by means of an induction coil probe calibrated to induced voltage levels in tissue.

In addition, the unit's internal circuitry allows ± 5% variation in pulse amplitude, width and rep rate before an alarm is activated.

The first coils delivered were of the single pulse type. By October '76 it was decided that half the coils be of the pulse train variety.

METHODS OF ANALYSIS OF EXPERIMENTAL RESULTS:

As noted above, Group III was exposed to PEMF continuously, employing one of two pulse parameters and the results of these groups were compared with those of Control Groups I and II in the following areas (post sacrifice at 28 days):
Radiographic
Histologic
Mechanical

X-Rays: Of bones from all three groups were obtained on a single industrial grade film for rough, visual quantification of bone density.

Histologic Analysis: In studies conducted in late '76 one rat tibia and os calcis were decalcified; serially sectioned in a longitudinal manner, and stained by hematoxylin and eosin. By keeping accurate count of the sections, comparable mid-sections of each sample bone are available for histologic analysis of internal and external architecture.

Mechanical Testing: The opposite tibiae and os calci are used for this analysis method. Care is exercised to keep the bones moist and properly aligned during mounting. All tests were completed within six hours after sacrifice in these initial studies.

Our three methods of analysis provided a cross-check system in evaluating the results of these studies. During the period from late 1976 through the first third of 1977 we were actively concerned with making certain that whatever the experimental results--our mechanical system of analysis gave results that "jived" with those observed in our other two methods of analysis--results that measured a clear phenomena in as sensitive and reproducible a way as possible.

Our first stage of mechanical testing was conducted on the Instron in the

Engineering School Laboratories at the downtown campus.

A preload of 2-5 pounds and a crosshead speed of 20 in. /min. was used in all studies. It was recognized that a more rapid rate of loading probably would yield more reproducible failure data, particularly in bones with a large amount of trabecular or porotic material. Thus, it was planned in late '76 to shift testing of this type from the Instron (with its slow rate of loading) to an MTS unit, which is capable of a much more rapid loading rate.

Os calci were mounted for 3-point bending failure and the proximal and distal tibial shafts were mounted for axial compression failure.

All post-fracture fragments were collected for dry, defatted, weight and ash weight determinations.

Beginning in January of 1977 the mechanical testing was conducted on an M. T. S. (Materials Test System), Series 810 which was installed in these laboratories at that time. The versatility and proximity of this system dictated that we discontinue the use of the Instron. This did not mean that the earlier data had been superceded; rather they have been embellished by increasing precision. The system was deemed necessary for these studies for the reasons mentioned above and to assist in exploring a new mechanical testing method to clear up the dilemma noted in late '76:

The osteoporosis model was continuing to give both histologic and radiographic evidence of a profound loss of cancellous bone in the tibial metaphysis. There appeared to be at least a five fold difference between Group I and Group II animals; it was difficult to explain the small (up to two fold) differences seen in compression and 3-point bending, breaking strengths. It seemed possible, therefore, that the cancellous loss was being "covered up" by less of a change in cortical bone.

As the above suggested that the spongy bone values were being "lost" in the more "rigid" cortical material encompassing it, this lab devised testing methods to assess only cancellous or cortical bone and by as early as April of '77 they had produced rather impressive results.

The new method of testing cancellous bone in the proximal rat tibial metaphysis was essentially the following: Resecting a fixed distance of the proximal tibia (0.4" in length), including part of the diaphysis. The specimen is mounted in a special holder by "cementing" the articular surface of the tibia (the knee joint surface) with fast-cure dental acrylic. This presents the open medullary canal to a circular plunger (.0 6 diameter, 0.4" long) mounted on the load cell head (100 lb.)

of the M. T. S. The plunger diameter is calculated to clear the inner cortical walls and is advanced toward the epiphysis through metaphyseal bone for a standard distance at a fixed speed. (A distance of 0.25"; at a rate of 0.625 in./min.) SEE Figures V R and VI R. Load vs. deformation time is recorded. The trace provided by this test demonstrates fracture of major trabeculae and a gradually increasing load as the mass of broken trabeculae at the tip are compacted against the epiphysis. (SEE Figure VII R.)

One of the additional benefits of the cancellous test is a nearly intact specimen, which after test, is processed for histology. The longitudinal sections, through the center of the plunger track, provide a magnificent view of what was tested and the size of "bolus" of fractured trabeculae, compressed at the tip of the plunger. The correlation between the mechanical test and the microscopic picture was impressive.

The cortical test employs a column of tibial diaphyseal bone which is "crushed" by a plunger of larger O. D. than the diameter of the tibial shaft.

In January of 1978 these labs began to examine a modification of the mechanical testing method for the tibial specimens, in order to study the effect of the epiphysis and ossification center on the test values. The usual test (described above) employs a 0.4" specimen with a 0.25" stroke to the plunger. The load developed in this system is a function of the precision of these figures and the amount of residual metaphyseal bone. It, also, may reflect the stiffness of the epiphysis and ossification center, which resist the bollus of fractured trabeculae being pushed ahead of the plunger. A study of a "push through" system was started which allowed the plunger to traverse the metaphysis, epiphysis and ossification center together with the articular cartilage. The mounting technique had been modified to provide a hole both in the dental acrylic and the specimen jig. With this system, it was planned to compare energy under selected and matched portions of the stroke distance/load curves. It was hoped that minor variations in specimen length could be eliminated with this approach, thereby increasing accuracy. The hope held for the "push through" test was misplaced and the final testing method duplicated the original M. T. S. protocol described above.

It is of note that previous studies reported in the literature of disuse osteoporosis in the rat employed ash weights and breaking strengths of whole bones as the major techniques to assay results and trabecular bone, the most "metabolically active" component, was not studied as an independent factor.

RESULTS OF RAT MODEL EXPERIMENTS

Late Summer '76

Expt.: Pilot Strain B Rats tested with single pulse "O" shaped coils. Instron testing system.

Results: 8 animals eliminated because of casting pressure problems (as discussed under development of rat model)

2 remaining Group III animals evaluated as follows:

Os Calcis Fracture: 54 lbs.
60 "

2 Group II Controls

Os Calcicis Fracture: 19 lbs.
32 lbs.

-Note modification of bone loss in this early study. Coils reducing to a remarkable degree the loss of bone induced by the "combined approach" method.

Late '76

Expt. First 30 rats subjected to the full protocol since August. Group III single pulse exposure, plus one pulse train exposed rat.

Results: Instron Analysis

Group I Cage Controls

Os Calcis Fracture:	57 lbs.	mean	6 animals
	42 - 85 lbs.	range	(12 specimens)

Note: a mean of 65-75 expected when mechanical testing perfected--results obtained when machine still being standardized. Low figures probably will be eliminated.

Group II Controls (Op/Casted)

Os Calcis Fracture:	32 lbs.	mean	3 animals
	19 - 43 lbs.	range	(6 specimens)

Note: a mean of 35-40 lbs. expected. Low values related to inadequate mounting.

Group III animals

Single Pulse Exposed

Os Calcis Fracture:	59 lbs.	mean	4 animals
	52 - 64 lbs.	range	(8 specimens)

Pulse Train Exposed

Os Calcis Fracture:	85 lbs.	(left)	1 animal
	96 lbs.	(right)	(2 specimen)

- Mechanical testing and x-ray analysis have demonstrated that this model produces a profound loss of bone in 28 days in the os calcis and the tibia.

Though the numbers are small, the tightness of the dats suggests strongly that the breaking strengths can be reduced by approximately 1/2 in 28 days in untreated animals. On the other hand, it appears that the breaking strength can be maintained in the normal range or, if one animal is a harbinger, it might be possible to increase breaking strength above normal. See results below. (This tendency was not confirmed in later studies using the pulse train.)

Dec. '76

Group III

Pulse Train Exposed

Os Calcis Fracture	96-155 lbs.	range	7 animals
			(14 specimen)

M. T. S. (Materials Test System) was installed in the laboratories. New mechanical testing methods were devised, as per earlier discussion, to test cancellous bone in this rat model: Jan - Apr. '77.

Note: Amplitude of induced voltage required to produce a therapeutic effect remains critically narrow.

- From both the cancellous and cortical bone specimens analyzed it has been seen that fields inducing 1.5 mv/cm of bone prevent a loss of mechanical strength, while fields which go above or below this level do not.

- These findings are in complete agreement with other studies of fracture healing models, bone graft models and treatment programs of patients with pseudarthroses and non-unions. All of these sources dictate an induced voltage between 1.2 - 1.6 mv/cm for bone.

May '77

Progress Notes: Pulsing electromagnetic fields have been shown by this contract to block completely disuse bone loss. Summary of findings to date:

Group II animals
- loss of spongy metaphyseal bone from the proximal tibia results after 28 days in a 90% reduction of ultimate breaking strength and
- 99.5% reduction in energy absorbed during fracture in this region.
- Breaking strength of cortical diaphyseal bone in the tibia is reduced 40% below normal litter mates (Group I).

Group III animals
- Exposure to the single pulse largely prevents this bone loss experienced by Group II animals
- Pulse train data from the end of '76 seem to indicate that pulse train exposure will double the breaking strength of both cancellous and cortical bone in Group III animals vs. Group I animals.

Equipment modification: New larger coils to facilitate animal placement and to assure uniform field densities across the whole body (via strict Helmholtz-aiding system)

- In these coils it was not essential to keep the casted limbs in an anatomical position, insofar as abduction or adduction was concerned.

- First Rat Series: Allowed to assume, randomly, any reasonable degree of abduction at the hips.

- First Rat Series Results: some animals failed to demonstrate (when exposed to effective fields) a protective effect (i.e., lost bone to untreated Group II levels), while the opposite limb demonstrated full to near full protection.

- These results led to the following:

Pilot Studies: Varying degree of abduction of rat hind limbs treated with coils to investigate the possibility that field vector lines, vis à vis the long axis of the bone being treated, may play a role in results:

Expt. animals: Strain B rats ordered as non-breeder, but some arrived pregnant. The supplier had problems and thus there was a combined effect of disuse and metabolic osteoporosis in some animals.

Abduction/Adduction of Hind Limbs: All of the treated tibiae in the following lists were casted at 23° $\pm$ 5° of adduction.

Except: 3 extremities placed at 45° and 90° of abduction produced peak loads of 0.5, 1.1 and 0.8 lbs.

4 extremities placed at 0° (abd/adduction) gave peak loads of 1.3, 1.0, 0.25 and 2.0 lbs.

Analysis: Some definite trends in the vector patterns are seen in the above results.

Reported results: peak loads in lbs. at the extreme of the plunger excursion in the cancellous bone of the proximal tibiae.

(M. T. S.)

Group I Cage Controls		Group II Controls	Group III Single Pulse Exposed
14.3		4.4#	
2.4	10.5	0.25	8.7
8.2*	5.9*	0.25	13.4
6.2*	16.2	3.7#	9.4
18.2*	19.0	2.0	15.3
19.0	16.5	1.3	11.6
22.0	15.4	1.1	5.2*
13.8	20.0	1.8	4.1*
18.5	5.7*	1.2	6.6*
7.3*	11.0	2.9	5.4**
9.5	7.0*	1.7	4.0**
7.0*	10.8	2.2	4.6*
14.1		1.0	
10.6		3.8#	
		0.5	

Symbols: * Breeder, recently pregnant or pregnant
** X-ray showed large bone mass, but mechanical test failure
# Inadequate cast immobilization

Note: From this data it appears that despite the unplanned pregnancy problem that the fields continued to demonstrate their protective effect, particularly with standardization of the appropriate field vector.

Fall '77 Experiments: To demonstrate the development of an improved model for producing rapid disuse osteoporosis in the rat tibia, employing the M.T.S. Strain B rats

Results: SEE Chart I R.

The major portion of trabecular bone in the proximal tibial metaphysis is lost within 28 days in the experimental groups (columns B and C) with this model:

- Group I Cage Controls (Free Roaming) produced peak loads (at a maximum plunger excursion) of 14.7 $\pm$ 3.8 lbs.
- Group II animals, which had been operated and fully casted, produced peak loads of 1.8 $\pm$ 1.3 lbs.

These results are significant at $P < .001$ and represent reduction in trabecular loading capability in excess of 80% in 28 days.

The mechanical testing results of control and experimental tibiae were compared with the radiographic and histologic appearance of longitudinal sections, taken through the center of the plunger track and found to exhibit a high degree of corre

Compare columns B and C: note increased efficiency of the model incorporating legs in plaster for producing a greater loss of trabecular bone ($P < .05$) than the model with the legs free.

The last column: a 260 gram rat appears to have a lower peak load than a free-roaming 300 gram rat from column A. Column A, Cage Control rats have a starting weight of 260 $\pm$ 10 grams but they tend to gain 10 grams per week; i.e., at the end of 28 days they weigh in the vicinity of 300 grams.

Chart I R, cont.

Summary: This unique method of mechanically testing trabecular bone can detect the extremely rapid and profound effect of disuse on cancellous bone. The analysis of data from columns A and B demonstrate that during this contract period a sensitive model system was developed to enable the survey of pulse effectiveness on the labile portion of the osseous system which may turn over rapidly and may account for the major fraction of negative calcium balance.

Expt.: To determine the effectiveness of PEMF produced by the single pulse or pulse burst signal in preventing bone loss in this rat disuse osteoporosis model. Strain A rats.

Results: SEE Chart II R which represents combined data from the "S" and "P" Series' Results.

The results of the Strain A series of animals, in which both the single pulse and pulse burst were applied, is presented in Chart II R. A value of 9.8 $\pm$ 3.2 lbs. was obtained for the free roaming controls (Group I) after 28 days. With the single pulse, the peak load was 8.3 $\pm$ 1.4 lbs. clearly, outside the range of the Group II controls, but, again, not quite equal to the maximal loads for Group I Cage Controls after 28 days.

The mean value of the single pulse exposed group is 85% of that for Group I Cage Controls. This apparent loss of complete effectiveness probably can be ascribed to the round coils--an issue to be checked by a later series of experiments.

The pulse burst did not appear to be as effective a deterrent to skeletal loss as the single pulse (albeit fewer animals in this group).

The analysis of peak load was taken from a discrete portion of the plunger stroke curves, thus the range of values for controls and free-roaming animals are more closely clustered than previous data (SEE (Chart I R) for the full stroke would indicate. There is, however, no overlap between the Group II and Group I values, with the means of the latter double that of the Group II animals.

For both the Strain A rats and Strain B rats (Chart I R) the use of the single pulse produced mechanical values which approximated normal Group I Cage Control values and which were statistically above the range of the Group II Controls.

Expt: "S" Series. Strain A Rats. Utilizing "punch out" method of mechanical testing (i.e., a selected portion of the plunger stroke curve was used for analysis: between .125" and .275".)

Results: Peak load values in lbs. (mean ± SD)

Group I Cage Control	Group II Controls	Group III	
		Single Pulse Exposed	Pulse Train Exposed
10 ± 2.3	3.4 ± 1.3	10	5.8 ± 2.1
n = 6	n = 9	n = 1	n = 5

Unfortunately, the number of single pulse specimens in this series was restricted because of the prevalence of periostitis secondary to cast pressure. Plus or minus values are not included for this reason.

It continues to appear, however, that the model is effective in producing a large decrease in trabecular bone and that the electromagnetic fields are able to modify this loss, with the single pulse being more effective than the pulse train. (These results are reported in Chart II in combination with the "P" Series Results.)

Expt: 100 Series. Strain A rats. Employing circular coils.

This series: to check the efficacy of coil design needed as a preliminary step to future studies on minimum exposure time. During previous phases of this contract, some differences in effectiveness of a given signal have been detected when various coil shapes and orientations were utilized.

Results: The final mechanical testing data from the rat tibiae in round coils had been analyzed and it confirmed a minimal effect of these fields, despite effective signal characteristics.

Notes: Use of various coil shapes. Other data from the various *in vitro* and *in vivo* phases of this laboratory's programs confirm the relative ineffectiveness of the round circular coils on cell function and repair patterns. These fields were designed to induce therapeutic voltages of 1.0 - 1.5 mv/cm in the system under study. In order to assure this level uniformly across the structure a Helmholtz-aiding system of coils is used. By serendipidy the first coils were square or rectangular in nature. These produced a uniform field pattern in the very center axis of the coils, but nonuniformity existed outside this axis. The circular coils were designed to eliminate the nonuniformity.

Unfortunately, these circular coils proved to be less effective for any given pulse characteristic. This behavior is seen most clearly and significantly in the ^{3}H Thymidine and ^{3}Uridine incorporation patterns in chick tibiae and chondrocytes and in the augmentation of healing in rat radial osteotomies: in all of these systems, rectangular coils appear to be more effective than square and square more effective than round.

Expt: "P" Series. Strain A Rats. Total body spica cast with hind limbs uncasted was used for Group II and Group III animals. Single Pulse.

Results: <u>SEE Table 1R</u>

- This series presents a highly significant set of results in which the mechanical values in the 10 Group III animals are almost identical to Group I Cage Controls. This result exists despite a mean body weight loss of Group III and Group II animals of 35 $\pm$ 18 grams and a gain for Group I Cage Controls of 12 $\pm$ 4 grams. Undoubtedly, these animals had not only disuse, but stress and endogenous steroids enhancing a catabolic phase. From previous charts and tables it can be seen that unlike some previous groups this series of cage control animals did not achieve peak load values above a mean of 9.6 lbs. (at the extreme of plunger excursion). This lower value probably results from a different breeder background of the animals which gained only 2-3 grams/week (to end at sacrifice at 272 grams), while previous groups gained 10-12 grams/week (Strain B) and ended at 300 $^+$ grams. Generally in Group I Cage Controls, there is a correlation between body weight and peak trabecular load, with higher weight animals producing the higher peak load.

If the values in this chart for the Group II animals are compared with those in previous reports, it also, will be seen that a mean of 4.0 lbs. (peak) is about double, some of the earlier values . Here it should be noted (as above) that the animals have a slower weight gain and probably, a slower rate of bone turnover. (Also, hind limbs were not immobilized.)

Expt: "Z" Series. Strain B. Rats. Total body spica cast (including hind limbs) Single Pulse and Pulse Train exposed Group III rats.

Results: <u>SEE Table II R</u>

- This study used the high weight gaining Strain B rats. The mean peak loads are 2 lbs. higher than in Table I R ($P < .05$) while the Group II Control mean values are quite low (1.9#). This latter result probably (as noted before) is a reflection of more complete immobilization and a higher rate of bone turnover.

The pulse burst was not at all effective in modifying the disuse osteoporosis, but the single pulse was. Effectiveness, however, seems reduced and reflects a change in the characteristics of the pulse (wider 385 msec and more rapid, 85 Hz) over previous studies of this generic pattern of the pulse. These changes were made in preparation to the attempts at reducing the treatment time (i.e., bigger pulse, bigger effect and less time). This reasoning appears to be erroneous.

For the future: narrower and slower pulses should be examined as a major step before further attempts are made to shorten the treatment time and power requirements.

In Summary, the major variables which affected absolute variations in results between the different series of experiments are as follows:

1. Pulse characteristics
2. Animal Strain
3. Immobilization technique
4. Testing method to evaluate results

Though the result values have been variable quantitatively, qualitatively all series produced the same results and trends.

Some further specific summary points:

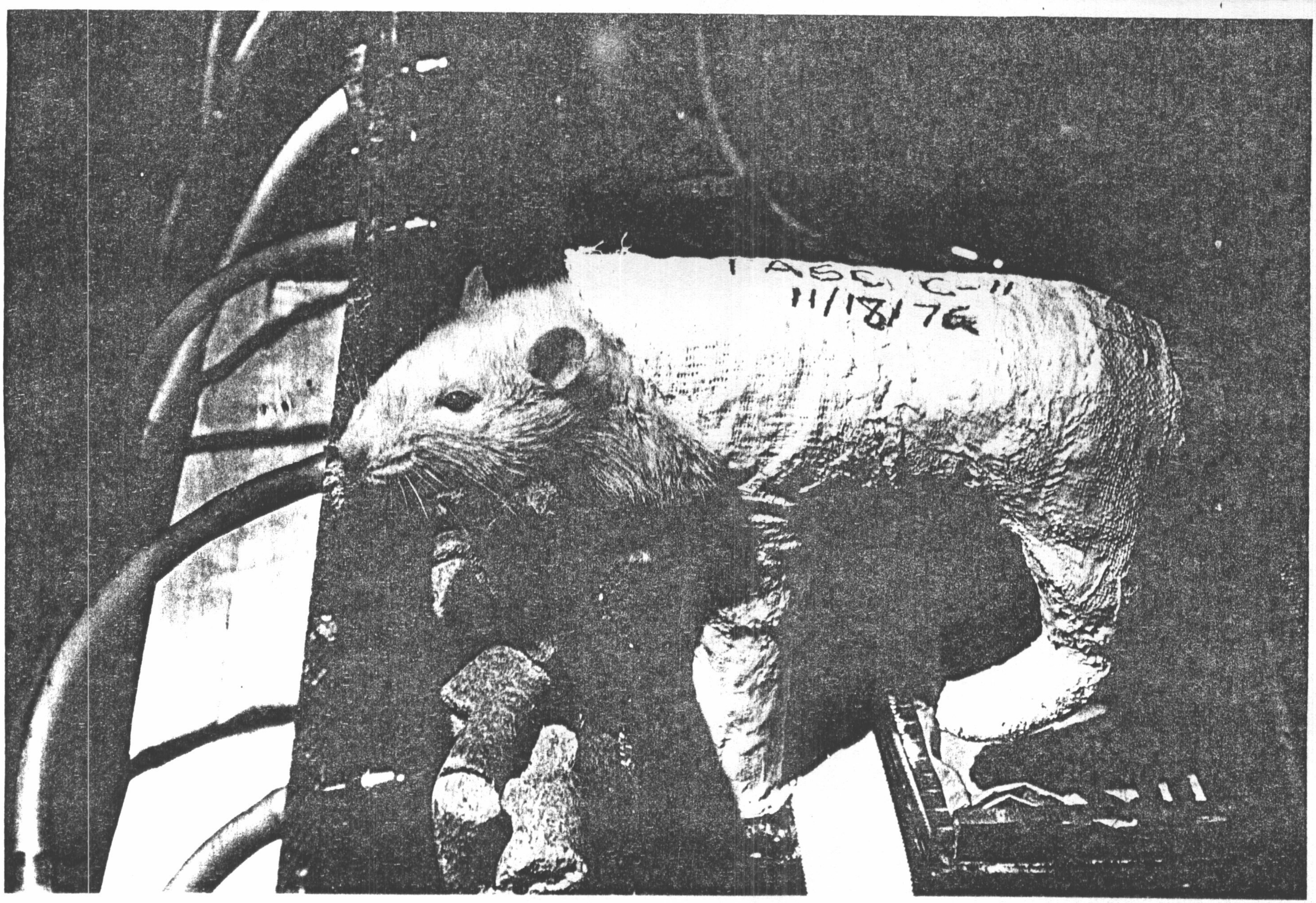
1. The loss of trabecular bone from the proximal tibial rat metaphysis following the "combined approach" rat model development has been documented radiographically, mechanically and histologically.

2. A new method of mechanical testing has proven to be a valuable and sensitive adjunct in detecting the extent of disuse osteoporosis in trabecular bone.

3. Exposing the "combined approach" rat model to PEMF for 28 days prevents disuse bone loss to a significant degree.

4. Apparently, electrical currents, induced exogenously by PEMF can be substituted for endogenous electrical currents, generated piezoelectrically by cyclic mechanical deformation, to control the cells which determine bone mass.

5. This surgically noninvasive method, ultimately, may have practical application in controlling bone loss in astronauts and in patients with local or generalized regions of bone resorption.



<u>FIGURE</u> <u>I R</u> Model for producing osteoporosis. Prior to application of plaster cast, the gastrocnemius, soleus and tendo Achilles were removed from both hind limbs. This system produces a profound loss of spongy (cancellous) bone in 28 days. This is our original "combined approach" model to which skeletal fixation pins through two proximal tail bones were added a few months later (See Figure II R). Certain series of rat studies ("P" Series) employed the total body spica with the hind limbs uncasted.

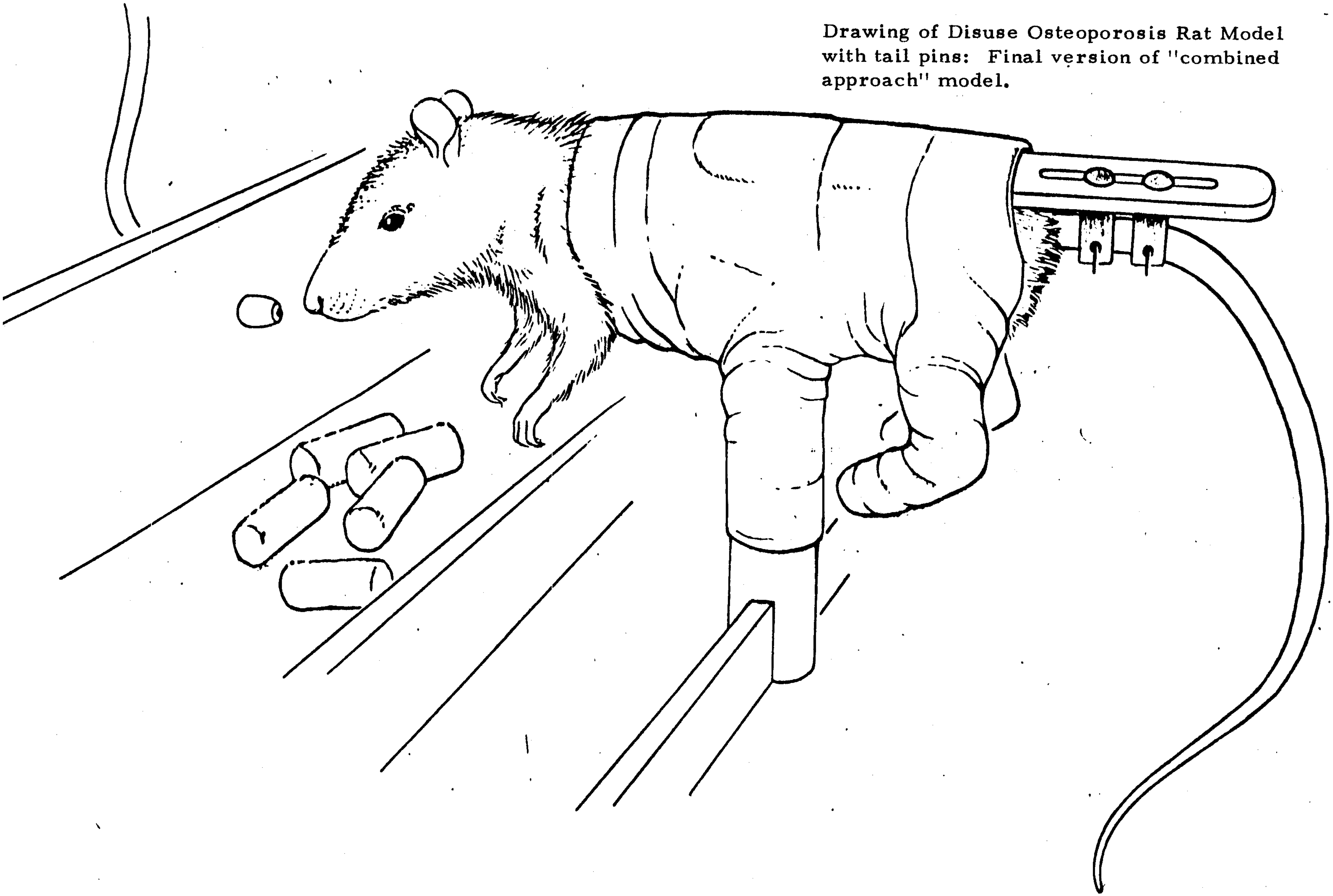


FIGURE II R

Drawing of Disuse Osteoporosis Rat Model with tail pins: Final version of "combined approach" model.

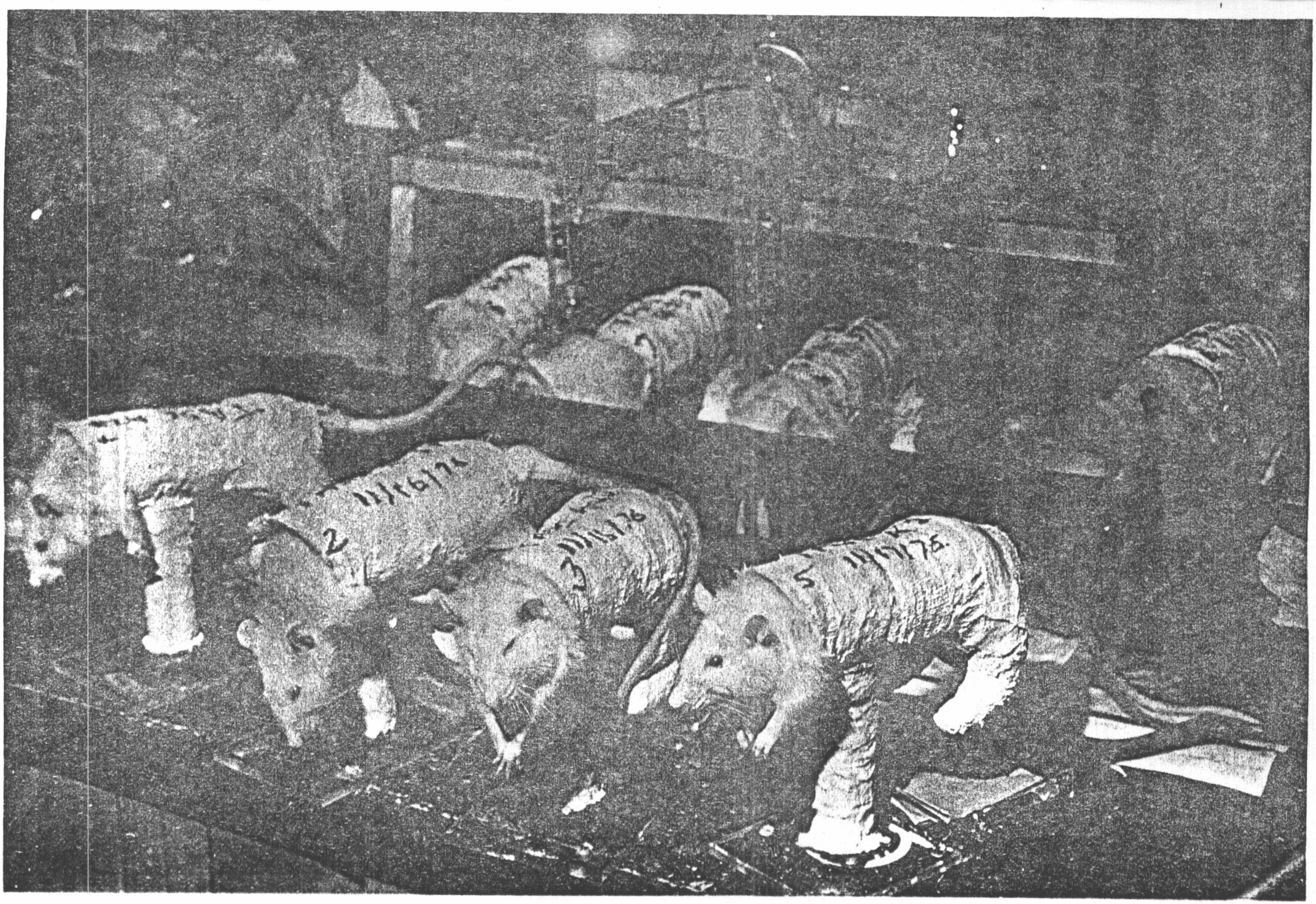


FIGURE III R   Operated and casted controls in front row, for demonstration purposes. Normally these are placed at a great distance from animals being treated in coils (back row).

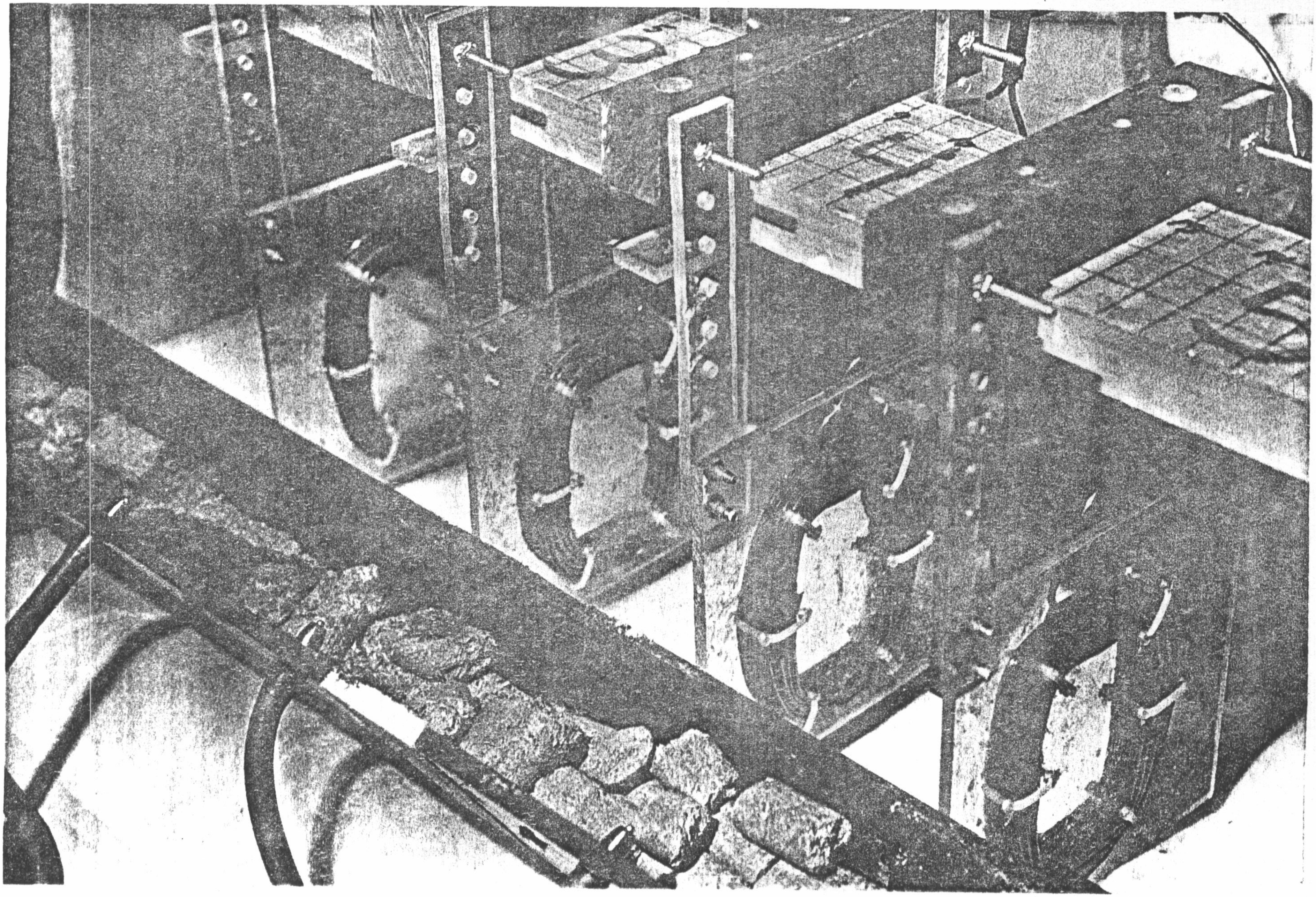
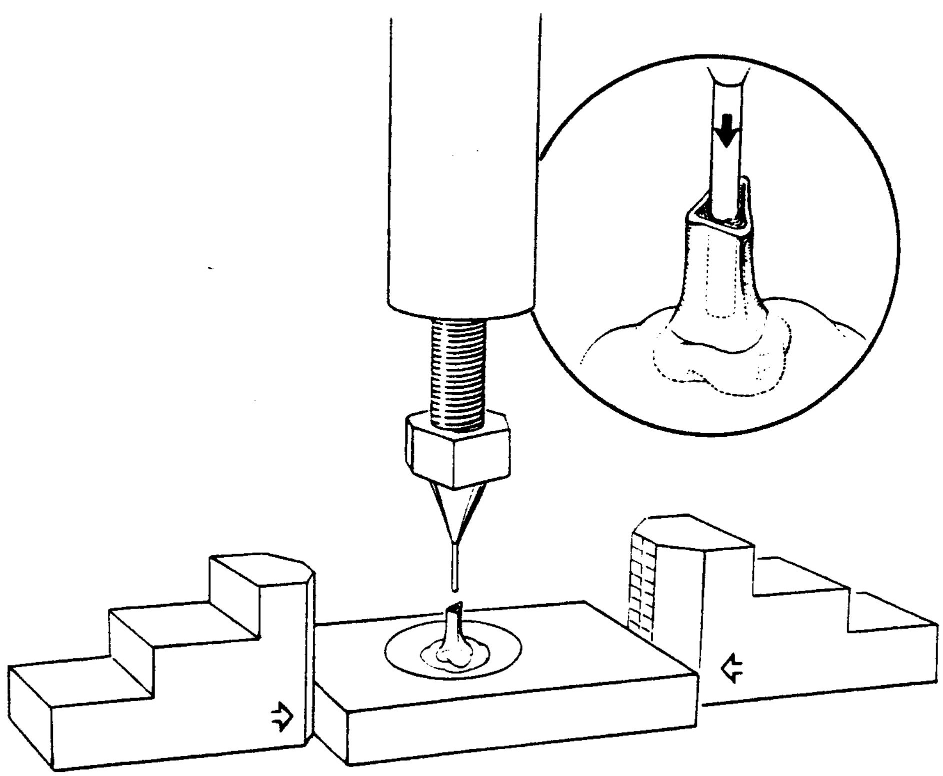


FIGURE IV R Electromagnetic coils used to modify disuse osteoporosis. The hind limbs are placed so as to be centered along the central (horizontal) coil axes. Treatment in this system begins immediately after operation and casting and continues for the entire 28 days of the experiment. Type of coil: Square coils (4" x 4"), used in the initial studies.

FIGURE V R

DIAGRAM OF MOUNTING FOR PROXIMAL TIBIAL SPECIMENS FROM RATS IN THE MTS MACHINE DURING MECHANICAL TESTING



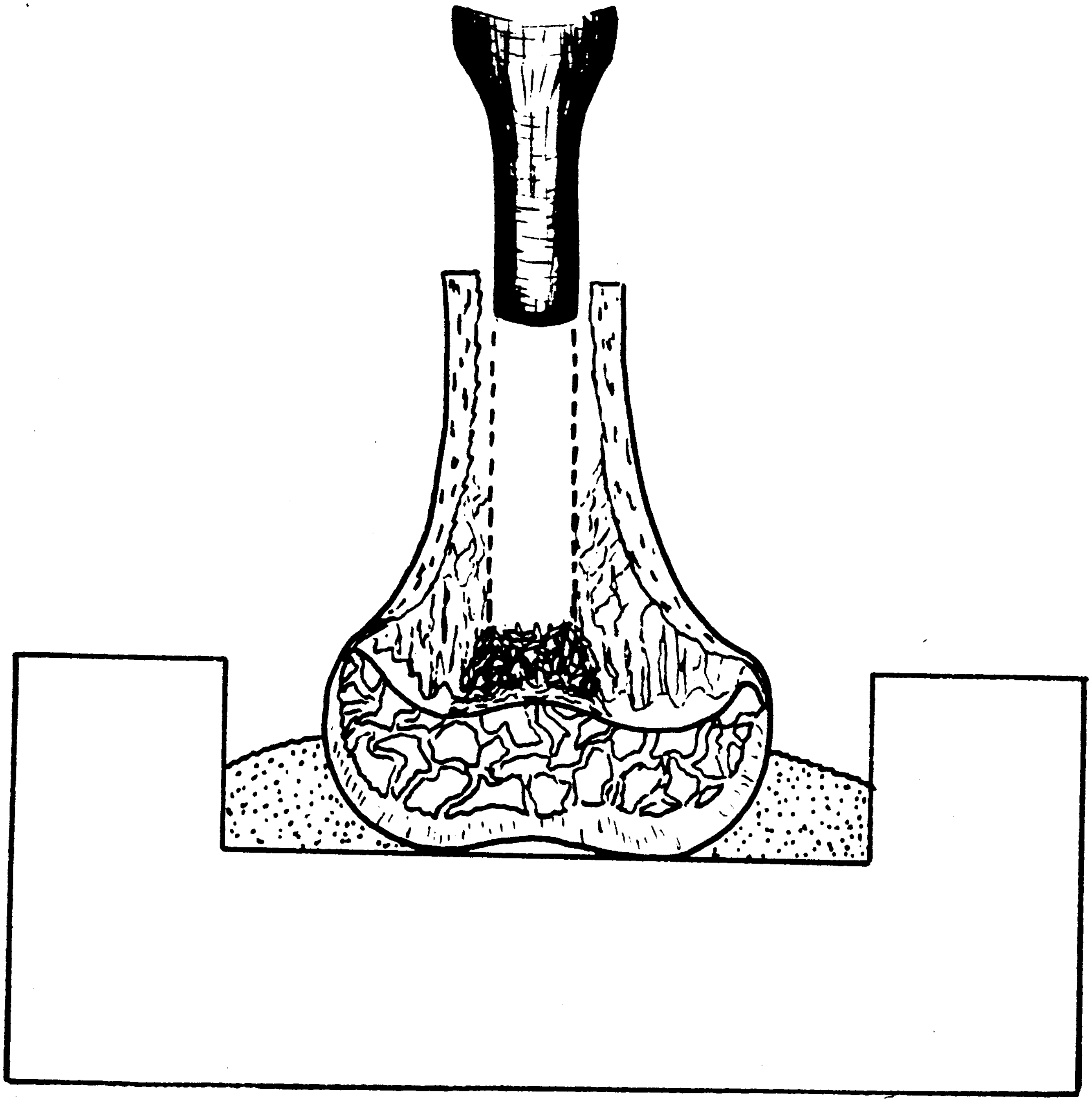
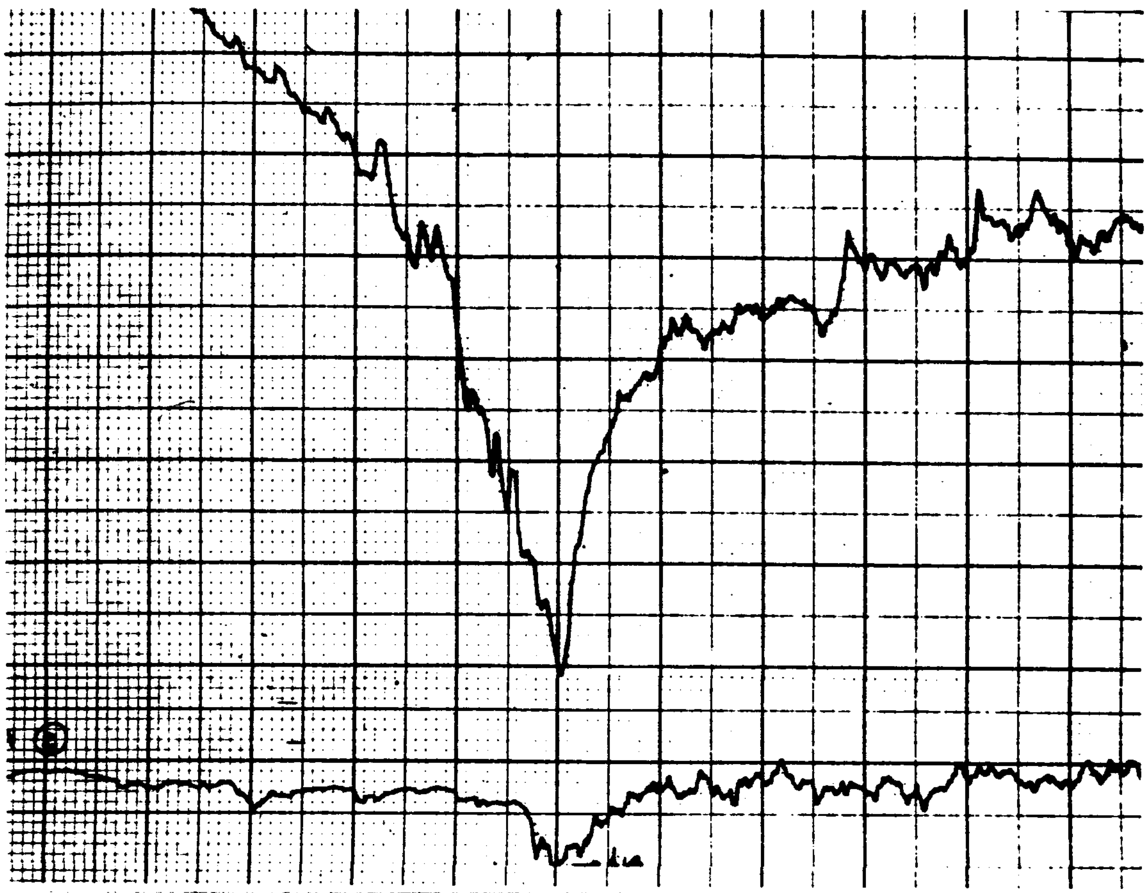


FIGURE VI R Method of mechanical testing of cancellous bone, proximal rat tibia. Specimen mounted in dental acrylic bond (stippled) to aluminum holder. All specimens of standard length. M. T. S. (Mechanical Testing System) plunger advances into the medullary canal at a standard rate and for a fixed distance, leaving a track of fractured trabeculae. Load cell records forces at plunger tip as it fractures trabeculae and compresses them into a mass just distal to the epiphyseal plate.

FIGURE VII R Load vs. Deformation Trace from M. T. S. for three typical tibiae. (See following page for details.)



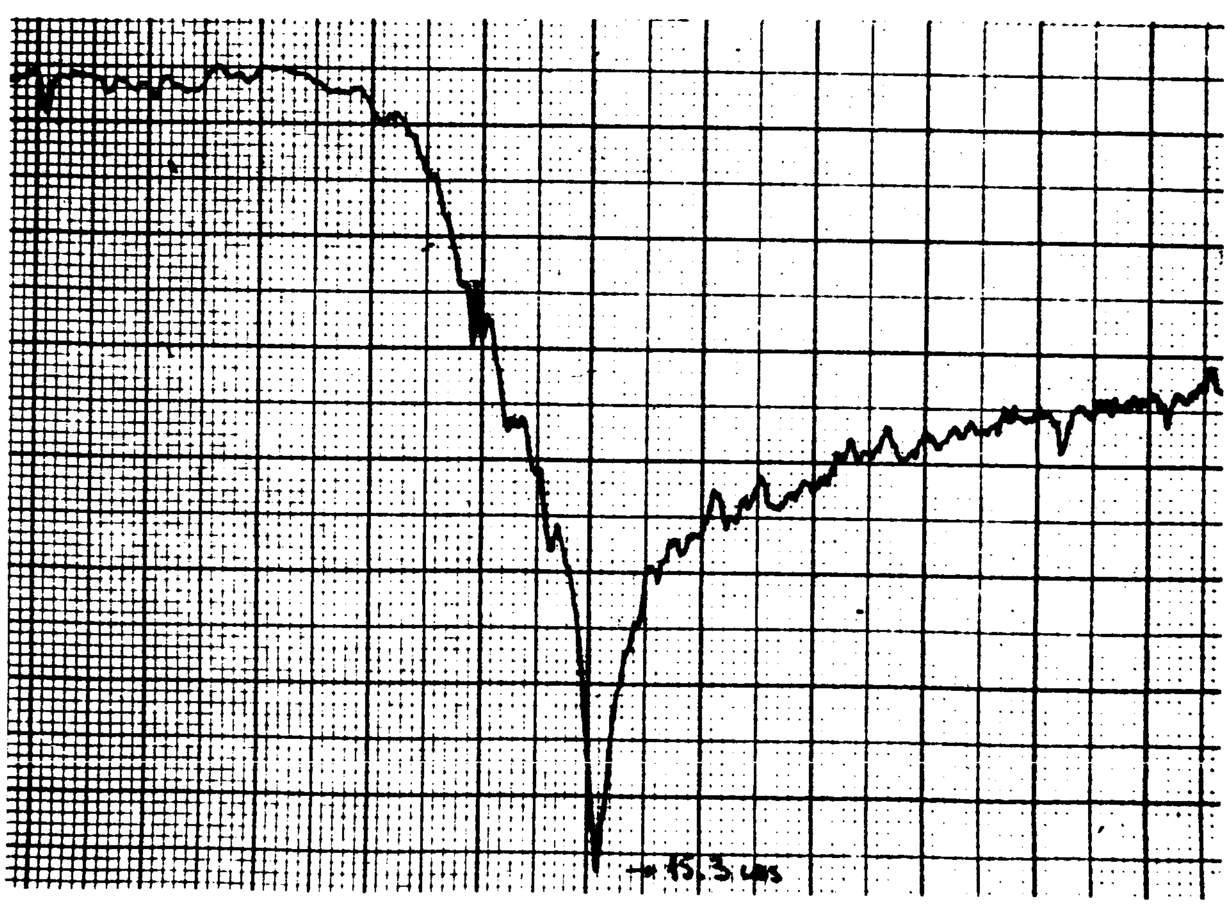


Figure VII R cont. Load vs. Deformation Trace from M.T.S. for three typical tibiae

1. (Top) X-Y plot of time (x-ordinate) vs. load (y-ordinate) for a normal standard laboratory cage living Strain B Rat (Control Group I)--260 gram starting weight. Sacrificed at 28 days. Peak load at the end of plunger excursion: 15.6 lbs.

2. (Center) X-Y plot, as in above figure but from a Group II Strain B Rat. Sacrificed at 28 days post operating/casting. Peak load is 1 lb.--compare with Group I animal in top trace.

3. (Bottom) X-Y plot for a Group III Strain B Rat of starting weight 260 grams. Sacrificed at 28 days post operating/casting/starting coil exposure. Note peak load equals Group I Control (top trace)--but the initial slope is less.

CHART 1R Peak Trabecular Loads ($\bar{x} \pm SD$) for Strain B Rats: MTS Data for Control Groups I and II, Group II with legs uncasted

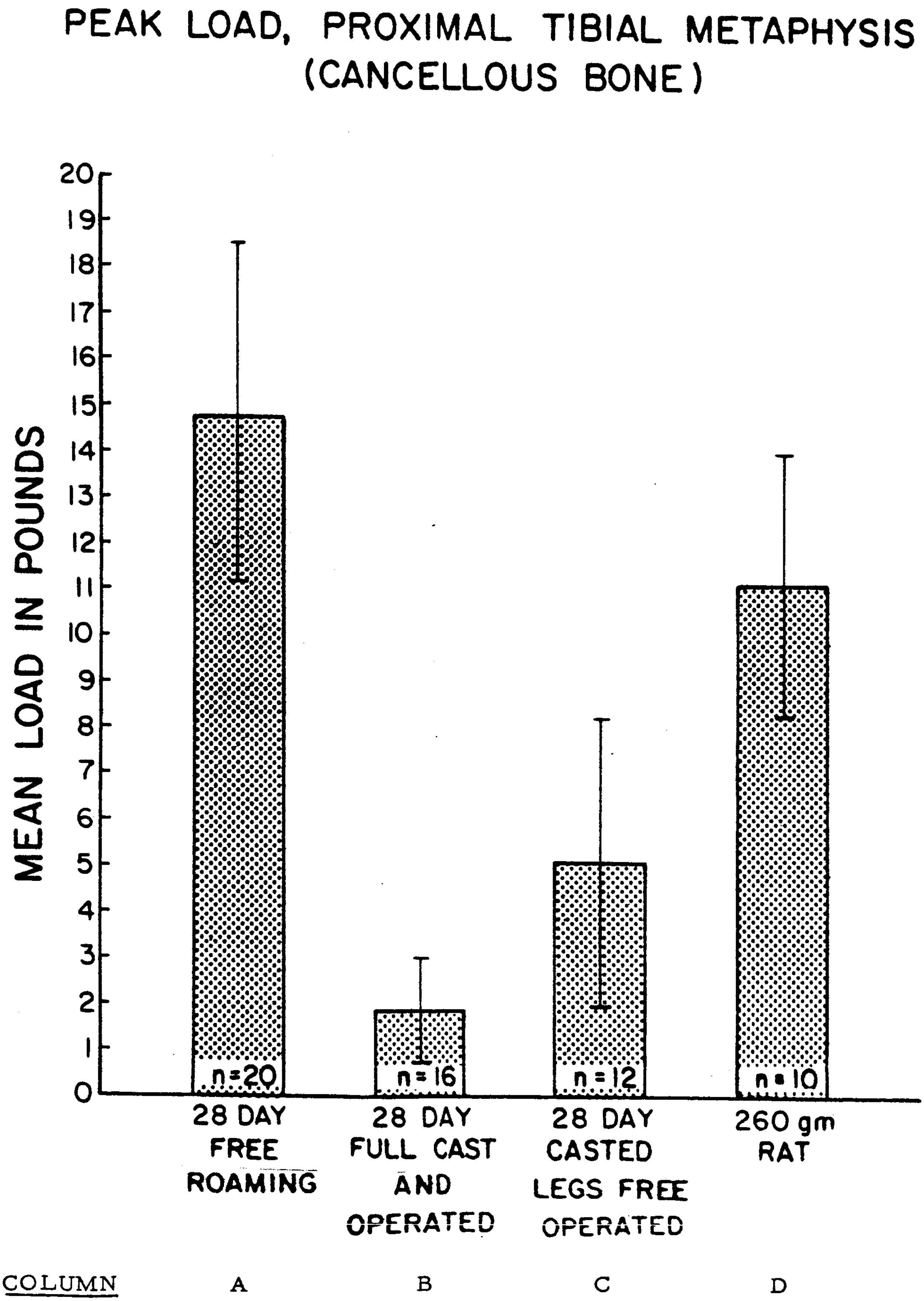
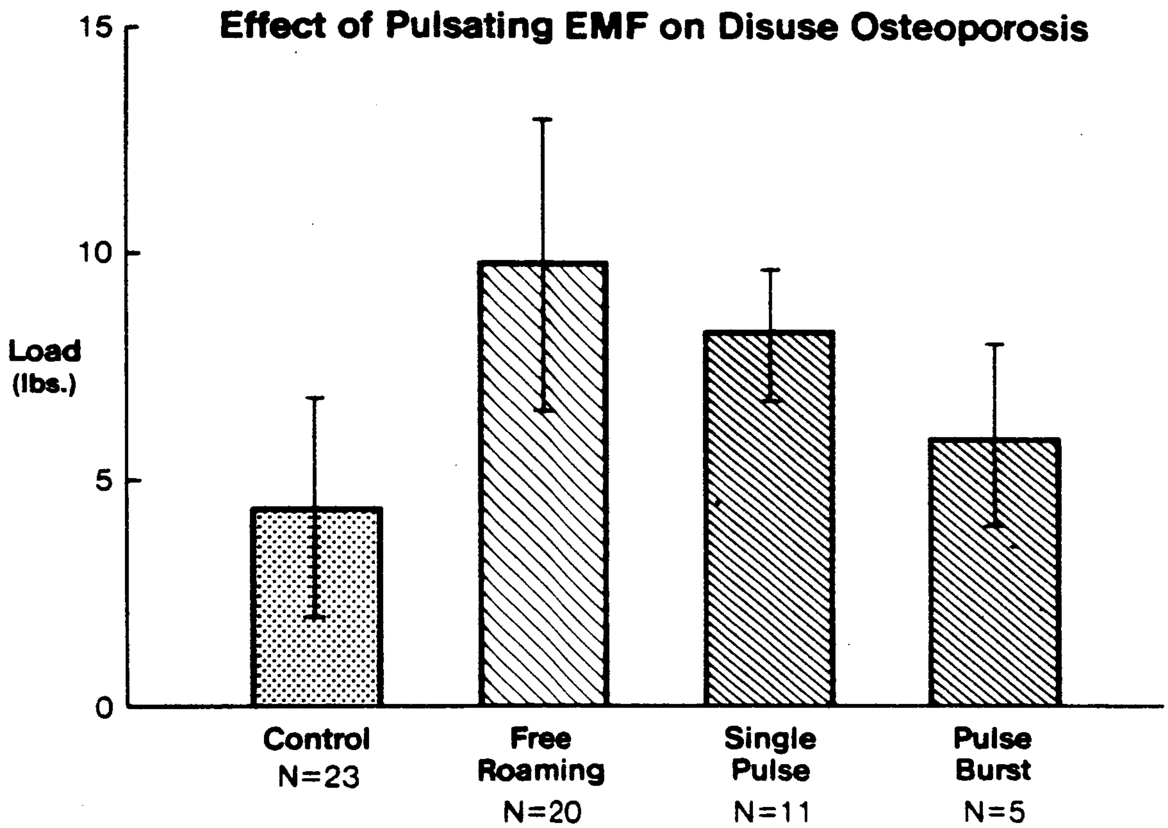


CHART II R Peak Trabecular Loads (x $\pm$ SD) for Strain A Rats: M. T. S. Data for Control Groups I and II and Group III Single Pulse and Pulse Burst Exposed.



Group II   Group I   Group III

COLUMN   E   F   G   H

Combined data from the "S" and "P" Series

"P" Series TABLE I R

PEAK TRABECULAR LOADS ($\bar{x} \pm$ SD) FOR STRAIN A RATS
MTS DATA FOR CONTROL GROUPS I AND II AND
GROUP III SINGLE PULSE EXPOSED

Experimental Animals		N	Peak Trabecular Load (lbs.)
Group I Control	Standard Laboratory Cage Living	14	$9.6 \pm 3.6^{*}$
Group II Control	Operated & Total Body Spica Cast (no hind limb cast)	12	4.0 ± 2.1
Group III Single Pulse Exposed	Operated & Casted as Group II	10	$8.1 \pm 1.7^{*}$

*Indicates significance $P < .001$ when compared to Group II

Round coils used (18 cm, Helmholtz-aiding)
MTS plunger deformation: .25"

"Z" Series

TABLE II R

PEAK TRABECULAR LOADS ($\bar{x} \pm SD$) FOR STRAIN B RATS
MTS DATA FOR CONTROL GROUPS I AND II AND
GROUP III SINGLE PULSE AND PULSE BURST EXPOSED

Experimental Animals		N	Peak Trabecular Load (lbs.)
Group I Control	Standard Laboratory Cage Living	38	$11.9 \pm 4.8^{*}$
Group II Control	Operated & Total Body Spica Cast (casted hind limbs)	15	$1.9 \pm 1.3^{*}$
Group III Single Pulse Exposed	Operated & Casted as Group II	7	$5.0 \pm 0.9^{*+}$
Group III Pulse Burst Exposed	Operated & Casted as Group II	6	$2.7 \pm 1.4^{*}$

*Indicates significance $P < .001$ when compared to Group I

+Indicates significance $P < .001$ when compared to Group II

Casting: 0° - 35° of abduction.
Round coils used (18.0 cm, Helmholtz-aiding)
MTS plunger deformation: .25"

OBJECTIVES: It is mandatory to prove the safety of pulsing electromagnetic fields for "whole-body" exposure as a prelude to extensive exposure studies in bed rest volunteers and, against the possibility that electromagnetic coils, ultimately, are to be used to prevent bone loss under conditions of recumbancy or weightlessness.

APPROACH: To this end the experiments employing pulsing electromagnetic fields (single pulse or pulse train signals) were initiated in December of 1976 to investigate the following:

1. Assay the ability of the field to increase skeletal mass above normal, as measured by ash weight/body weight ratios.

2. To assay possible "toxicologic" effects of chronic field exposure. For this phase of investigation a variety of tissues other than bone were studied from each of the several field strengths and vectors (pulse shape remaining the same). This study was possible with the 4 and 6-level plexiglass "mouse manors" used beginning in December of 1977. The study of the effects of uniform and non-uniform fields (employing fields with proven therapeutic capacities) was conducted on a variety of organs: brain, heart, kidney, adrenals, liver, spleen, pancreas, gut, ovaries and testes using standard histopathologic techniques.

3. To assay possible teratologic effects of chronic field exposure: mothers and offspring were studied. The offspring were studied particularly for growth patterns during this chronic exposure and for successive generation effects both in and out of the fields. Skeletal mass and organ profiles along with growth curves provide the data base for these studies.

EXPERIMENTAL ANIMAL

Swiss Webster Mice supplied by Camm Laboratories

Sex and pregnancy status specified for each experiment.

All animals are given a 14-day acclimatization period after arriving at these laboratories before being assigned to control or experimental groups. During this period the animals are separated by sex and observed to assure they are disease free and that the females are not pregnant. (One exception was the 4-level mouse manor study where the females were mated at Camm Labs and delivered at these laboratories one week prior to delivery.)

EXPERIMENTAL DESIGN

Control animals: Standard laboratory cage living cages: 12" x 5" x 7" (L-H-W)

Experimental animals: animals that were exposed to PEMF: 24 hours/day. Pulse characteristics are described in rat model section.

Sacrifice dates:

20, 28, 40, 42 or 60 days: indicated for each experiment

With the exception of the first 3 series of mice studies (Table 1 M), the mice experiments were conducted using the following system:

New restraining cages (mouse manors) were designed and built to permit the checking of a whole series of field strengths and vectoral patterns simultaneously. (These studies began at the end of 1977.)

one female & 2 males are assigned to each level or cage after acclimatization.

<u>Group I</u> Control — are housed in standard sized laboratory cages as described earlier. (Referred to as Control Cage animals in the charts for this section.)

<u>Group II</u> Control — are housed in mouse manors without coils. Referred to as Restraining Cage Controls (or rest. cage controls in some charts).

<u>Group III</u> — are housed in mouse manors with a pair of Helmholtz-aiding coils affixed between floors as described in the text that follows. Animals are exposed continuously to either the single pulse or pulse train as specified in each experiment.

<u>Mouse Manor system</u>: consists of either 4, or later, 6 levels of plexiglass trays for residency, stacked one on top of the other. Each tray, or level, is a 5-sided box (19 x 19 x 6 cm) with the "ceiling" provided by the main lucite shelf, tray support structure.

Each tray may easily be removed by sliding it horizontally from its shelf.

Group III Mouse Manors

- a pair of Helmholtz-aiding coils (8" x 8") are mounted with their long axes parallel to the table top: one between floor one and two (attached to the "ceiling" of floor one) and the other between floors two and three (attached to the "ceiling" of floor two).

- Signal characteristics: single repetitive pulse (4-level manor)
  repetitive pulse burst (6-level manor)

- These trays were designed to constrain the mice to the region within the coil "windows", i.e. intercoil distance of the coils.

- This arrangement allows one tray of mice below the coils, one inbetween, and two (or four) above the coils.

- A design of this type permits the survey simultaneously of field effects ranging from homogenous, 1.5 mv/cm induced to nonhomogeneous 0.15 mv/cm induced.

- SEE Figures 1 M and 2 M for a diagrammatic representation of the 4 and 6-level mouse manors, respectively, with the amplitude ranges provided for each tray level.

Both male and female mice are used in these studies, including pregnant or mated females, to study the effect of this system on conception, embryogenesis, growth and development.

RESULTS OF SWISS WEBSTER MICE EXPERIMENTS:

3 Series of Experiments on the Effects of Whole Body Field Exposure on Skeletal Mass (SEE Table 1 M)

- Mouse manors were not used for these experiments.

- After sacrifice at 28 days the animals were weighed, major soft parts removed and the remaining carcass (including all of the skeleton) ashed.

The only few clearly significant animal results were from the single pulse exposed animals in Series 1. These few animals, 2.9 - 3.4 ash weight/body weight %, were exposed to lower field strengths than normally used in this program and were in fringing regions. These results demand a more definitive study be done to see if this increase of 1-20% in ash weight could be reproduced.

Results from Series 2: It appears from these results that control values are nearly identical with those reported for Series 1's controls. In addition, it appears that the pulse train exposure (at least with the chosen pulse parameters) does not affect the total skeletal mass in these animals in 28 days to any significant degree.

Results from Series 3: are only marginally significant.

Summary of results for these three series: The data seem to suggest that adult mice exposed to fields for 28 days may slightly increase their skeletal mass in otherwise normal animals.

Both the gross physiological evaluation and the histopathologic survey of the major organs of all three series of animals indicated no abnormalities.

MOUSE MANOR STUDIES

Objective of studying the single pulse in this first mouse manor study:

This signal was assayed under the experimental conditions described on pages 2M and 3M because it seems to have the greater effect in retarding or preventing disuse osteoporosis in the rat.

- The first pregnant mice, mated at Camm Labs and delivered one week prior to term, were put into the three experimental Groups I, II and III described earlier as a prelude to mating and rearing F^1 through F^4 generations, chronically exposed to the field.

- It's important to note that control and experimental animals fall into one of the following categories (this applies to this study and the following study using the pulse train):

1. Animals sacrificed at 40 - 60 days postnatally.

Single Pulse
Mouse Manor Study, cont.

2. Animals remaining in designated cage or restraining manor level to initiate successive generation.

3. Test animals removed to a control condition following some period of test system exposure.

4. Animals maintained in designated cage or restraining manor level for long term surveillance study.

Results: Results from this study are presented in Charts I M, 2 M and 3 M, and Table 2M.

CHART IM

The F^3 generation survived only 20 days (by design). Despite the fact that these mice were markedly immature their ash weight/body weight % was above the normal range for the Group I cage control animals.

From this chart it is evident that the only animals which had a statistically significant increase in inorganic % over the Group I cage controls, as well as the Group II restrained controls, were the animals on levels 1 and 3 The induced voltages at these two levels are 0.8 and 1.1 mv/cm, respectively.

Furthermore, since the animals on level 1 have the major portion of their body above the floor level, the voltage at body level, is probably an average of 1 mv/cm. At level 4 with an induced voltage of 0.2 mv/cm there was no statistically significant increase over cage controls but, there was over the controls in restraining cages.

Summary: Ash weights from these animals seem to indicate that as in the first 3 series described earlier--exposure to fields may increase skeletal mass slightly in otherwise normal animals, particularly when the single pulse is used (compare notes under pulse train manor study).

Histopathology No significant abnormalities were noted. One exception concerns the loss of trabecular bone from the metaphysis of Group II mice (both single pulse and pulse burst studies). This was not seen in single pulse treated animals, but remained an observation in pulse burst treated mice.

<u>CHARTS 2M and 3M</u>: Growth Curves (age vs. weight gain)

From Chart 2 M it is clear that there are differences in weight gain over time between Group II Controls and Group I Controls. The Group II and Group III animals are identical in their weight gain patterns in the F^1 and F^2 generations (Chart 3 M). Thus, the restraining cage seems to be the important variable rather than the PEMF exposure affecting weight gain.

The growth rates of Group III mice continued to fall within the standard deviations of the Control Group II animals through the F^3 generation (not shown on Chart 3 M).

These two charts demonstrate that the weight gain, as a function of 40 days for treated animals, falls within the range of the controls (both Group I and Group II). There are individual variations in the treated litters which might be significant if larger numbers were involved, but this is not seen as a compelling problem. The uniformity of birth weights for the Group III animals should be noted.

The F^4 litter, which had a normal birth weight was flooded out by a break in the watering system, so we lost the continuity of succeeding generations in which field bred and raised animals were used to produce offspring which, in turn, spent their entire lives in the field until further breeding.

<u>TABLE II M</u>: Litter sizes for Group I, II and Group III animals.

From this chart it should be noted that the numbers of offspring (as was mentioned above for birth weights) for field exposed animals show a much narrower range than those of controls: More Uniformity of Litter Size in PEMF Exposed Mice.

STUDY SUMMARY: These skeletal mass, teratology, "toxicology" studies were terminated in the F^4 generation. From this set of studies was achieved a line of animals exposed continuously to the fields in excess of 5 months without any evidence of major abnormality, unless an increased ash weight, more uniform birth weight and litter size may be considered aberrant.

Mouse Manor Study II Employing the Pulse Train

These experiments are almost identical in nature to those described for the Single Pulse Mouse Manor Study and begun in the fall of 1978. The main differences from this earlier study are the pulse characteristics (noted in Figure 2 M), the use of a 6-level mouse manor (vs. 4) which provides a different set of induced voltages surveyed per level (See Figure 2 M) and a sacrifice date at 42 days.

Objective of commencing study of the pulse train in the mouse manor system: To assess the pulse train (burst) signal (5 msec wide 15 Hz) as a baseline for future studies designed to decrease effective exposure time to an absolute minimum. Also, it was initiated to define the issue of the increased skeletal mass as a function of growth in both "signals".

Initial Results: Results from this study are presented in Charts IV M and V M and Table III M.

TABLE III M : Comparison of sacrifice weight, ash weight and ratio of ash weight to body weight of F^1 generation of mice from Control Groups I and II and the Group III pulse burst exposed animals.

In this series there was only one group with a statistically significant increase in percent inorganic over cage restrained and none over cage controls. These results should be compared with those seen for the single pulse exposed animals (SEE Chart I M). Also, this one significant group occurred in animals with a markedly reduced body weight, so the result is open to serious question.

CHART IV M AND V M: Growth curves (weight vs. age) for first generation litters born and raised exposed to PEMF (pulse train) and Control Groups I and II.

SIGNIFICANCE OF MOUSE MANOR STUDIES USING PEMF FROM SINGLE PULSE OR PULSE BURST SIGNALS:

The results presented for these studies in growing mice buttress the data from the rat model program in several ways:

1. First, the effects of pulsing electromagnetic fields on skeletal tissues are probably not species specific.

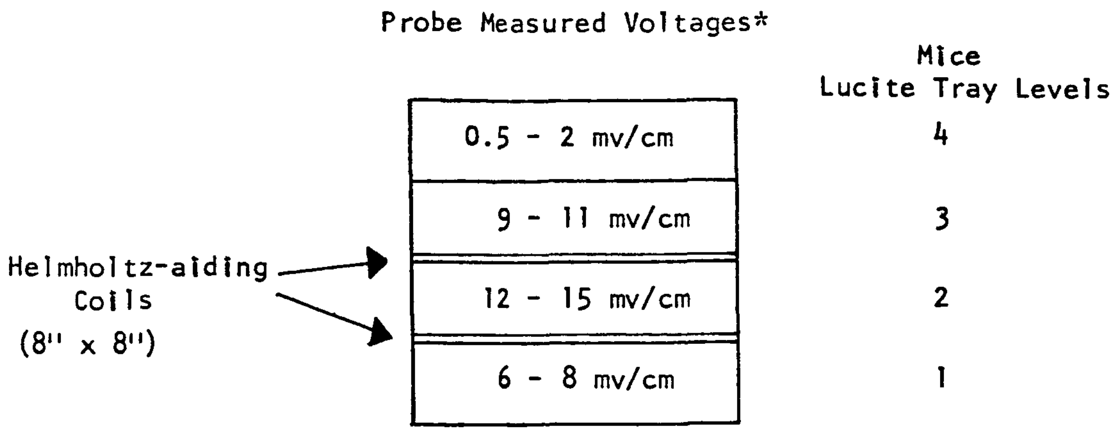
2. At or near the therapeutically induced voltages, no deleterious effects have been documented in large numbers of mice over significant periods of whole body exposure. The fields, therefore, appear at this point in time to be reasonably safe.

3. The mouse results indicate, again, the single pulse is more effective than the pulse burst for modifying skeletal mass.

4. It appears that a critical induced voltage level must be reached in this system (assuming appropriate pulse shape) in order to produce effects. This "threshold" pattern seems similar to that observed in the clinical use of pulsing electromagnetic fields for non-unions. In the latter application, most rapid and beneficial effects on healing are achieved in the range of 1.0 - 1.5 mv/cm of bone. Below and above these levels it is difficult, if not impossible, to document changes in the state of the non-union either by radiographic or by clinical means.

FIGURE 1M

DIAGRAMMATIC REPRESENTATION OF
4 LEVEL MOUSE MANOR USED FOR
ANIMALS EXPOSED TO PEMF (Single Pulse)



* These coil probe measured voltages are 10 times higher than the induced voltages measured in tissues. The higher of the two values are center field and the lower, the peripheral field (but within the inter-coil distance of the coils).

Thus, tissue induced voltage ranged from a high of 1.5 mv/cm at the center of level 2 (flanked by the coils) to a low of 0.05 mv/cm at the periphery of level 4, some 6 inches away from the nearest coil face.

GENERIC SINGLE REPETITIVE PULSE CHARACTERISTICS:

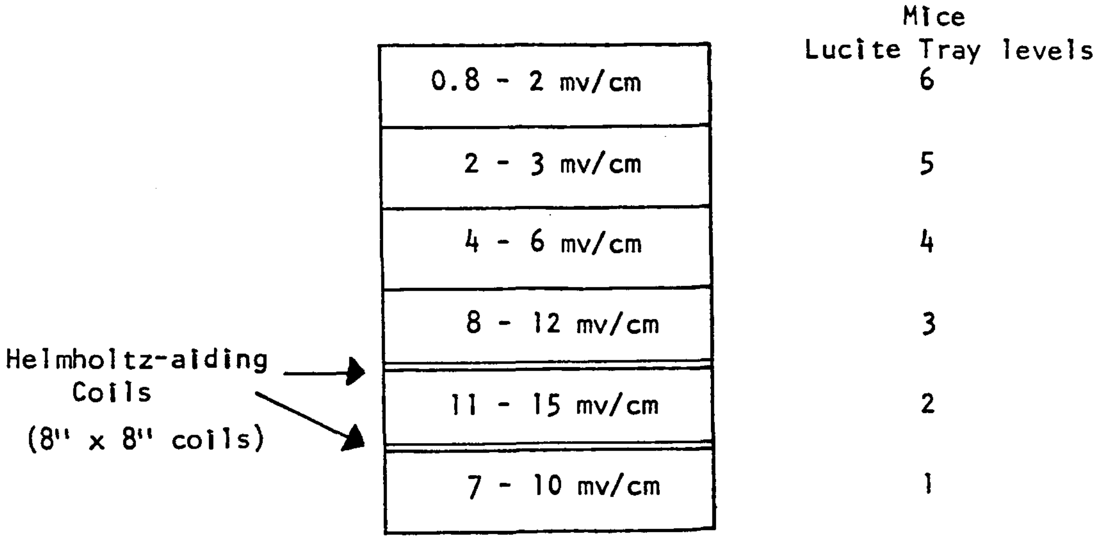
0.2 - 0.4 msec positive going

12 - 15 msec repetition rate

FIGURE 2M

DIAGRAMMATIC REPRESENTATION OF
6 LEVEL MOUSE MANOR USED FOR
ANIMALS EXPOSED TO PEMF (Pulse Burst)

Probe Measured Voltages*



* These coil probe measured voltages are 10 times higher than the induced voltages measured in tissues. The higher of the two values are center field and the lower, the peripheral field (but within the intercoil distance of the coils).

GENERIC REPETITIVE PULSE BURST CHARACTERISTICS

4.0 - 6.0 msec positive going

50-90 msec repetition rate

CHART I M

ASH AND BODY WEIGHTS of Successive Generations of Control Groups I and II and Single Pulse Exposed Group III Swiss Webster Mice

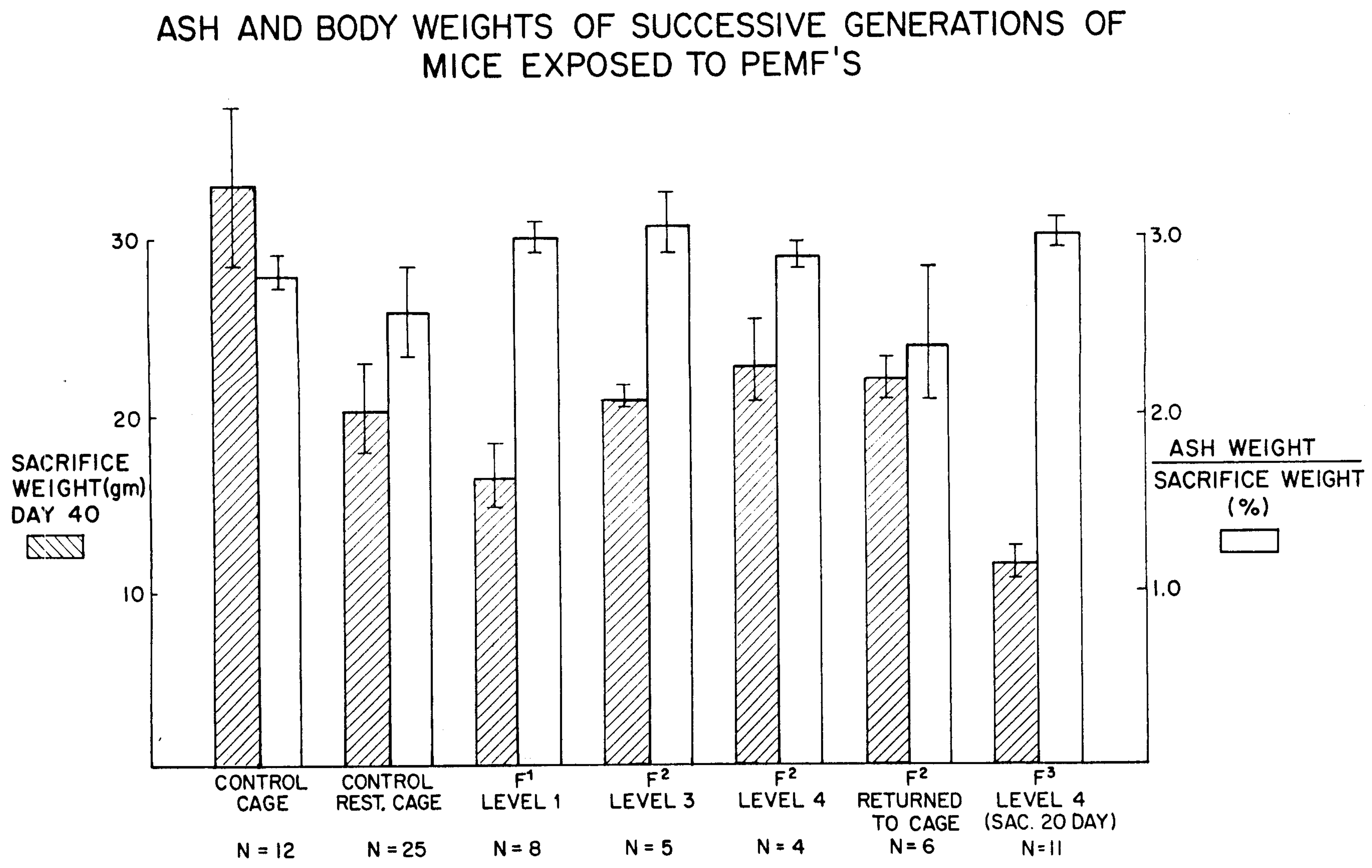


CHART II M

GROWTH CURVES for Control Groups I and II
(for single pulse mouse manor study)

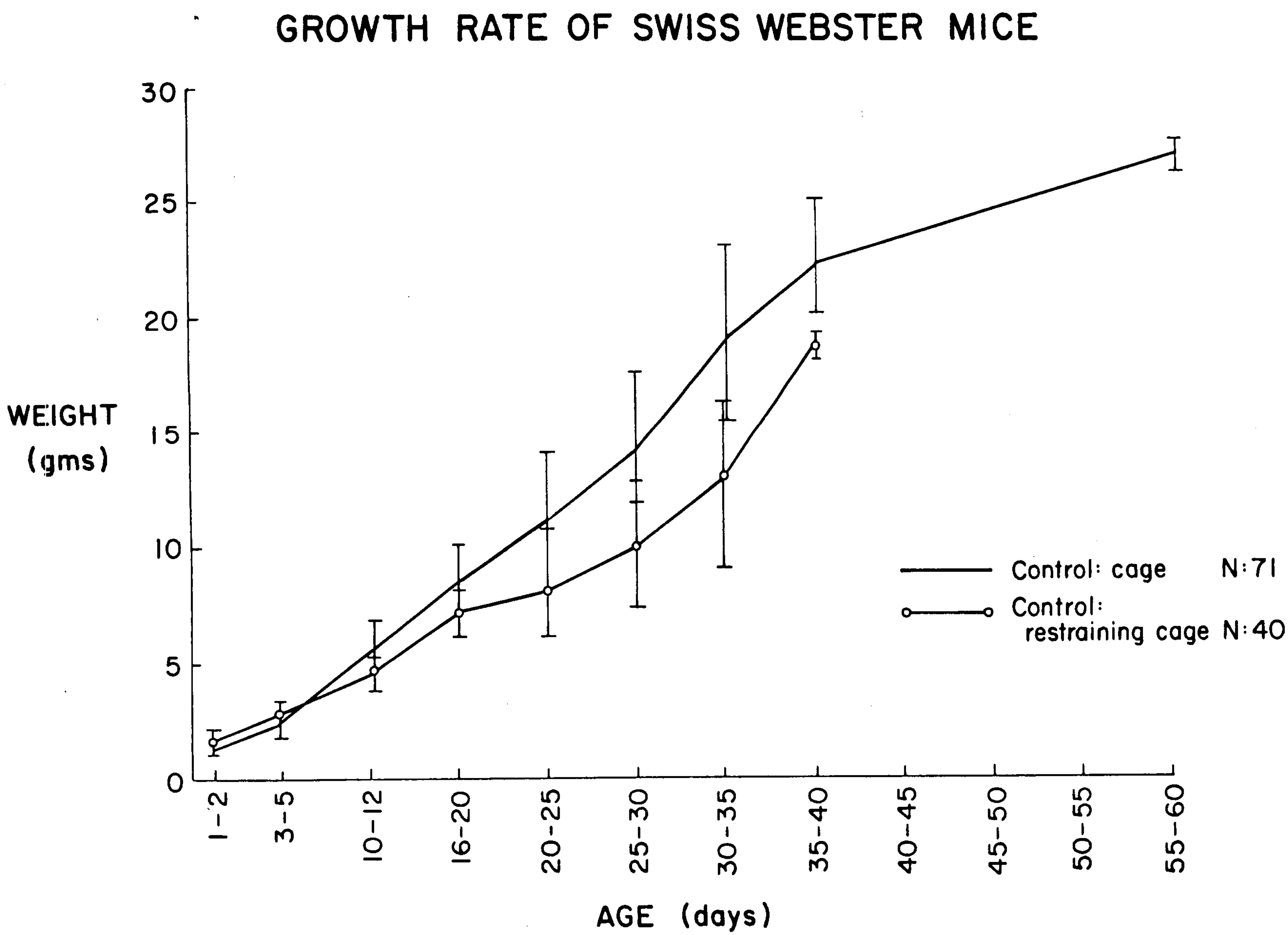


CHART III M

GROWTH CURVES for Swiss Webster Mice
Group III Single Pulse Exposed 4-level mouse manor

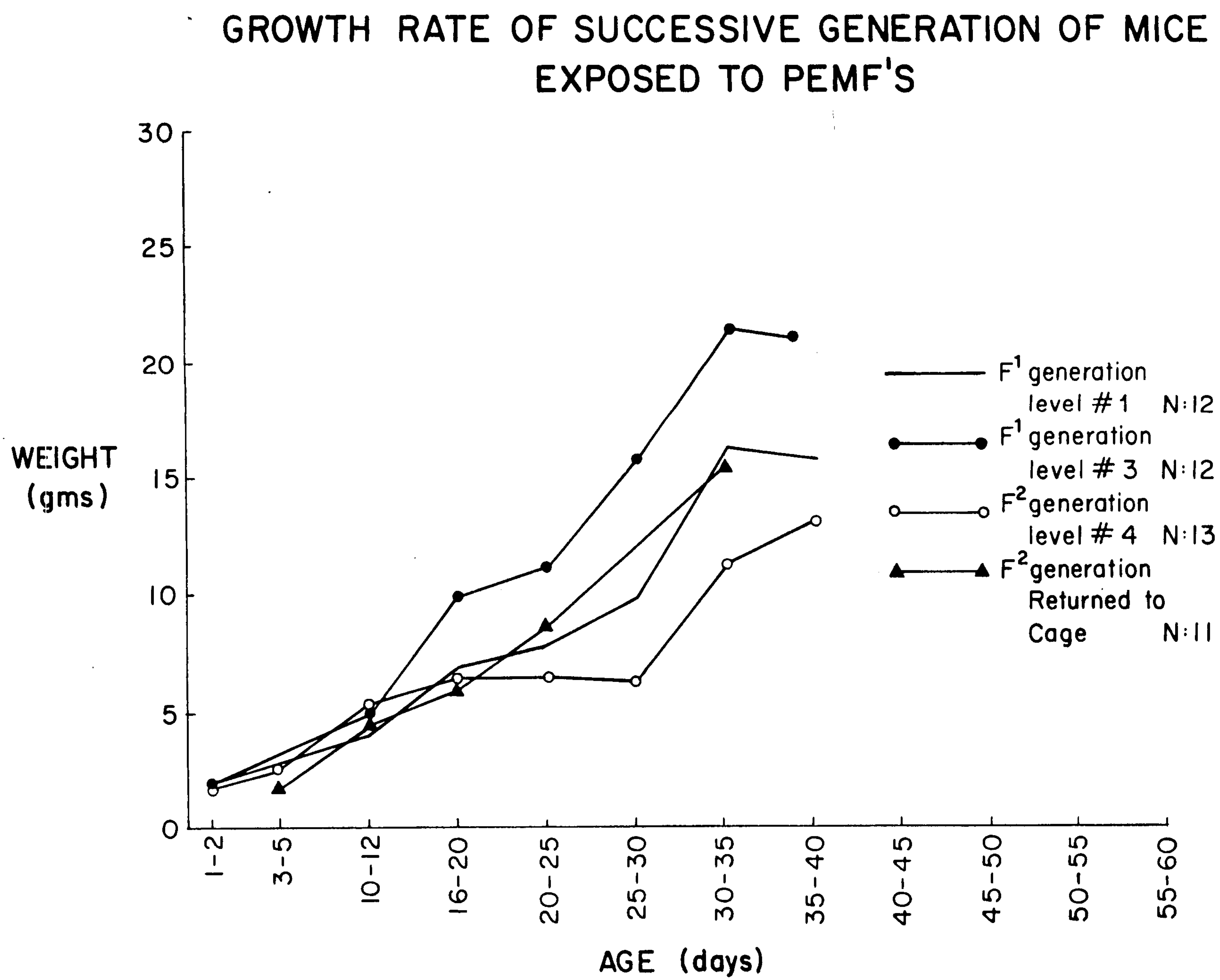


CHART IV M

GROWTH CURVES for Control Groups I and II
(for pulse burst mouse manor study)

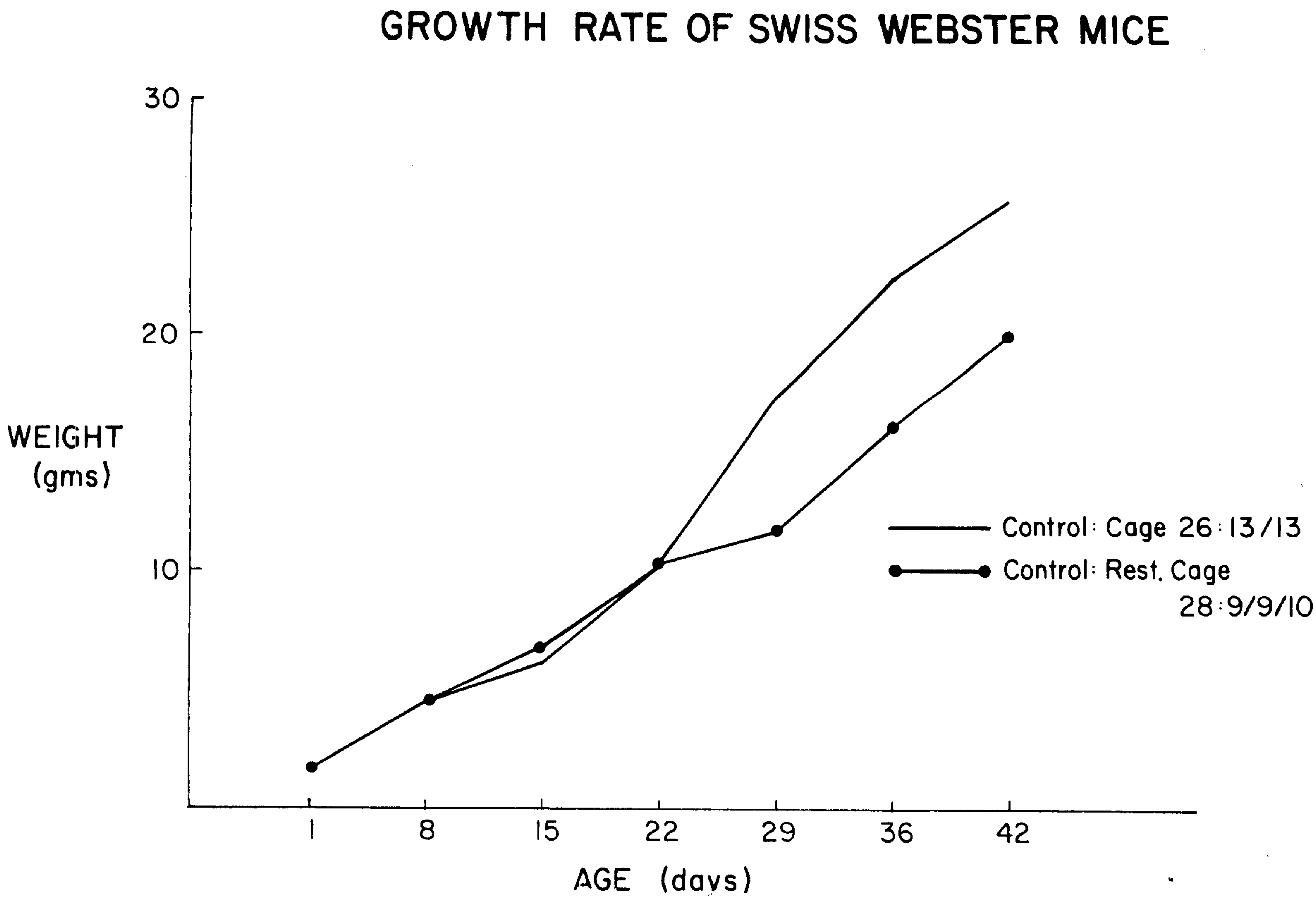
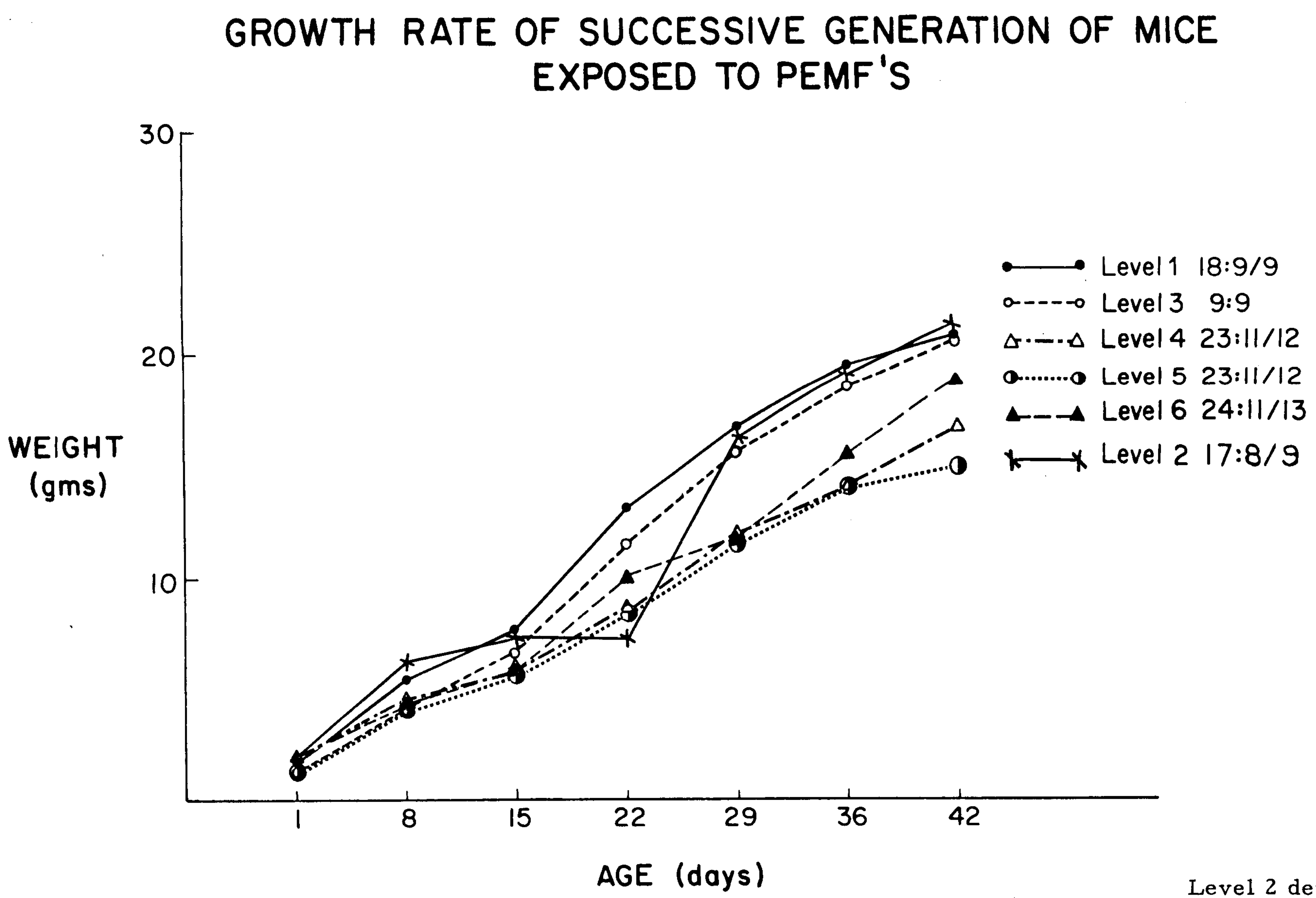


CHART V M

GROWTH CURVES for Swiss Webster Mice
Group III Pulse Burst Exposed
Successive Generation 6-level mouse manor



Level 2 deviation at Day 22 due to flood.

Swiss Webster Mice

TABLE 1 M

EFFECTS OF WHOLE BODY FIELD EXPOSURE ON SKELETAL MASS

ASH WEIGHT/BODY WEIGHT % ($\bar{x} \pm$ SD) IN 3 SERIES OF EXPERIMENTS

	Experimental Animals (all female, 40 gm.)	N	Ash Weight/Body Weight % + (mean ± SD)
SERIES 1	CONTROLS	8	2.7 ± 0.1%
	SINGLE PULSE EXPOSED	16	2.9 ± 0.35% *
SERIES 2	CONTROLS	8	2.67 ± 0.26%
	PULSE TRAIN EXPOSED	16	2.71 ± 0.15%
SERIES 3	CONTROLS	8	2.79 ± 0.21%
	ALTERNATING PULSE TRAIN/SINGLE PULSE **	16	2.94 ± 0.25%

*4/16 mice fell well above the range for the controls with values of: 2.9, 3.1, 3.2 and 3.4%.

**Cage fitted with "pulse train" coils for 4 days; with "single pulse" coils for 3 days: the schedule repeating weekly for 4 weeks.

+All animals sacrificed at 28 days.

Teratology/ Toxicology Study
Swiss Webster Mice

TABLE II M

LITTER SIZE DATA

Expt. Group	Litter Description	# of Litters	Litter Size Day 1 total ($\bar{x} \pm$ SD)	Litter Size Day 40-60 total ($\bar{x} \pm$ SD)
Group I Control	Standard Laboratory Cage	8	71 (8.9 ± 3.2)	50 (6.3 ± 2.0)
Group II Control	Restraining Cage (mouse manor)	4	40 (10 ± 2.9)	38 (9.5 ± 3.1)
Group III Single Pulse Exposed Mouse Manor	F^1 Generation Level #1	1	12	8
	F^1 Generation Level #3	1	12	5
	F^2 Generation Level #4	1	13	7
	F^2 Generation To cage living	1	11	8
	F^3 Generation Level #4	1	11	11 (after 20 days)

TOTAL Mice Population (Day 1) : 170

Teratology/Toxicology Study
Swiss Webster Mice

TABLE III M

COMPARISON OF SACRIFICE WEIGHT, ASH WEIGHT AND RATIO OF ASH WEIGHT TO BODY WEIGHT OF F^1 GENERATION MICE: CONTROL GROUPS I AND II AND GROUP III PULSE BURST EXPOSED MANOR

Experimental Groups		N Equal # males/females	Sacrifice Weight gms.	Ash Weight gms.	Inorganic %
Group I Control	Standard Laboratory Cage	14	24 ± 3*	.63 ± .09*	2.7 ± .1
Group II Control	Restraining Cage (mouse manor)	12	19 ± 2	.50 ± .02	2.6 ± .1
Group III Pulse Burst Exposed Mouse Manor	Level # 1	10	20 ± 2	.55 ± .03	2.8 ± .2
	Level # 2	4	22 ± .5	.61 ± .03*	2.8 ± .2
	Level # 3	10	19 ± 2	.52 ± .06	2.8 ± .1
	Level # 4	12	15 ± 2*	.44 ± .05	2.9 ± .2*
	Level # 5	14	15 ± 2*	.41 ± .07*	2.7 ± .3
	Level # 6	12	18 ± 2	.50 ± .05	2.8 ± .2

* Indicates significance P<.001 when compared to Group II mice.

OBJECTIVE: To study the effects of PEMF on disuse osteoporosis in the mature rabbit.

SPECIFIC ISSUES AND APPROACH: These pilot studies on the disuse osteoporosis model chosen for the rabbit are to focus on the following issues:

1. To demonstrate that electromagnetic field modification of disuse osteoporosis is not species specific.

2. Analysis of bone based on contralateral controls in the same animal (made possible in the rabbit model vs. rat model due to increased size of the animal).

3. The mature rabbit should provide some insight into the role, if any, of growth and remodeling which always occurs in the rat, secondary to its life long open epiphysis. (Note: Rat model investigations have indicated large differences in disuse osteoporosis between a rapidly growing 260 gm animal and a slowly growing 260 gram animal. Thus, results of rabbit model studies would be important to confirm this tendency.)

EXPERIMENTAL ANIMAL

New Zealand White Rabbits, female, approximately 1-2 years old.
mature animals demonstrate closed epiphyses upon x-ray
immature animals demonstrate open epiphyses upon x-ray
Supplier: Camm Labs

EXPERIMENTAL DESIGN

Control Animals: Live in Standard Laboratory Cages (approx. 25" x 23" x 15"; L W H). One rabbit per cage.

Experimental Animals: Rabbit Model for Disuse Osteoporosis
This model involves a "combined approach" similar to that of the rat model:

1. Operative procedure: Section of the sciatic nerve and tendo Achilles (SEE Figure 1 Rb)

2. Casting procedure: whole body spica
   nipples to toes (bilateral)
   - casted animals are suspended on a pedestal (3" in diameter, 6 in. high)

One casted limb serves as a control (some shielded with conel metal)

Experimental limb:
- fitted with a pair of 18 cm or 4" x 4" Helmholtz-aiding coils (as indicated/study)
- these coils are mounted to the surface of the cast.
- In all experiments the treated limb was exposed to PEMFs for 24 hrs. /day.
- PEMFs were either single repetitive pulse or repetitive pulse burst (as described in rat model section).

Experimental Results
Method of Bone Analysis:
radiographic, histologic and mechanical testing.

Summary of Evolution of mechanical testing analysis of bone in this rabbit model:

Animals from Pilot Study #2 analyzed:

Notes: Heterogeneity of trabecular distribution in the os calcis tibial metaphyses and distal femoral metaphysis is impressive on gross and radiographic evaluation of these regions from normal mature animals.

Plunger testing in a variety of specimen orientations: Further demonstrated the heterogeneity noted above and made it clear that this highly satisfactory test in the proximal tibial metaphysis of the rat was likely but inappropriate for the rabbit.

Normal or longitudinal "crushing" of whole bone: again, seemed to dilute effects to be observed when trabecular bone is the main focus.

In April '78 preliminary studies of radial splitting (cone or wedge) test of the rabbit os calcis seemed to overcome these problems:
- It appeared that any major loss of trabecular structure in this region removes the "tie rods", allowing early "cortical-cancellous"

failure of the external shell of this bone.

- This test reflected more of a cortical component than desired, but it was a reproducible method.
- By June '78, results with this testing method appeared to be sufficiently sensitive to detect minor to moderate modifications of trabecular structure in this bone.
- July '78: This test system for the os calcis demonstrated that a plunger with a .07" diameter and a .02" length backed by a 30° cone will give both trabecular and radial splitting data which are highly reproducible on contralateral bones. The values are ± 5% right vs. left.

RESULTS OF RABBIT MODEL PILOT EXPERIMENTS:

- It was established that the rabbits tolerate plaster immobilization well.

- It was established that coils can be mounted externally on the cast to treat one extremity; with the other serving as control.

PILOT STUDY #1. 6 rabbits confined in plaster spica cast to determine if they tolerated 40-60 days confinement in spica following operation.

PILOT STUDY #2. Rabbits casted only (as above) with no operative procedure performed.

- Attempts planned to mobilize these animals after removal from casts at the end of 6-8 weeks.

- After rehabilitation period of 6-8 weeks, the bones (hind limb) were subjected to mechanical and histological analyses.

Results: Attempts were unsuccessful in mobilizing any animal after prolonged casting with or without subjection to the operative procedures.

For the future: More time and finances needed to develop necessary rehabilitation aids. This phase of the protocol discontinued.

PILOT STUDY #3.
1st Group Exposed
to PEMF (Single Pulse)

4 rabbits subjected to operating/casting

- 3 of 4 animals had one limb fitted with a pair of 18 cm (bent oval) Helmholtz-aiding coils mounted to extensor/flexor surfaces of the cast. Other limb served as a control* Sacrifice: 35 days.

- 4th animal: Days 1-35: post op/casting, no coils
Days 35-70: Single Pulse coils applied to one extremity
Sacrifice at day 70.

*Note: Because of the configuration of the field "blow-out", the contralateral limb induced voltage values are four orders of magnitude below the treated and these can be reduced further by two orders of magnitude by Conel metal shielding. The latter will only be employed if proven necessary by a comparison of the untreated control limbs with limbs of totally control animals.

Results

Radiographic analysis: substantial differences between treated and control extremities.

Mechanical testing using the punch approach to the rat tibia (modified for the rabbit femur) failed to demonstrate the differences noted radiographically. (Technical failure of test method.)

Histologic analysis: confirmed radiographic results.

Further notes:

- the 70-day rabbit demonstrated the most profound differences (right vs. left):

1. Coil treated extremity's os calcis: appeared almost normal in its density (by "eyeballing" it) and in its trabecular widths and resorption cavity counts.

2. Untreated limb: showed marked loss of trabecular material and cancellization of the outer cortical shell.

- Histologic feature of all 4 animals with treated os calci (three, 35-day and one 70-day animal):

Pilot study #3, cont.

- the cement lines in the treated os calci are increased in number and in thickness
- the significance of this observation is not clear, unless it has to do with slower turnover rates of old material, coupled with surprisingly increased accretion.

For the future: more labeling studies need to be done to confirm these points.

PILOT STUDY #4

To provide additional pilot data on new mechanical testing method: in order to answer the question of effectiveness in detecting trabecular bone loss in the shortest time.

4 experimental animals, 2 cage controls

Experimental animals: one limb of each affixed with 18 cm coils:

Single Pulse: 2 extremities (1 mature, 1 immature animal)
Pulse Burst: 2 extremities (1 mature, 1 immature animal)
This provided 4 control limbs, 4 treated limbs.

Sacrifice of all animals at 35 days.

Results:

A. Immature Animals.

1. radiographs at 35 days showed (post sacrifice) extensive osteoporosis in the os calci of both the treated and control sides.

2. mechanical testing using the radial splitting method demonstrated a

75% < in ultimate (failure) load when the control (op/casted) os calci were compared with 4 normals from cage control rabbits of the same age.

Therefore: it appeared that this method of testing was satisfactory and probably would give a high level of correlation with radiographic and histologic data.

Pilot study #4, cont.

B. Mature Animals.

Control os calci demonstrated a

30% < in failure loads, but ranges fell almost within the variations of loads derived from normal cage control adult os calci.

It seemed apparent, therefore, that the adults must be kept longer in the cast (probably 70 days) before sacrifice and testing in order to establish the kinds of data necessary in a study which will employ fewer animals than the rat study.

Summary: There is a strong suggestion that animals with open epiphyses have a greater rate of bone turnover and are more "sensitive" to disuse situations than mature animals.

Mechanical test values indicated that neither the pulse train nor the single pulse modified the osteoporosis for this model system in this time frame.

Improvements needed: These results don't mean that the rat results cannot be confirmed in rabbits: coil design and placement still is critically important in the success of this modality (even given an effective pulse). Round coils were chosen before we began to focus on this factor and the casting method placed the os calci at such an angle to the field that the induced voltage was below the low (induced) threshold of 1.0 mv/cm. All of these deficiencies can be corrected.

PILOT STUDY #5

SEE Table 1 Rb

2 controls: Standard Laboratory Cage Living

Experimental Animals: 5B, 7B, 5S and 7S: operated and casted

Pilot Study #5, cont.

Expt. limb: Exposed to PEMF

Pulse Burst Exposed: limbs 5B right
7B left

Single Pulse Exposed: limbs 5S right
7S right

Control limb: shielded with conel metal (signal inside attenuated--have them 10^{-3} over the contralateral PEMF exposed limb)

Rabbits 5B and 5S were subjected to above conditions for 5 weeks .

Rabbits 7B and 7S were subjected to above conditions for 7 weeks.

Results: SEE Table 1 Rb.

SIGNIFICANCE OF RABBIT PILOT STUDIES:

These studies have served to demonstrate:

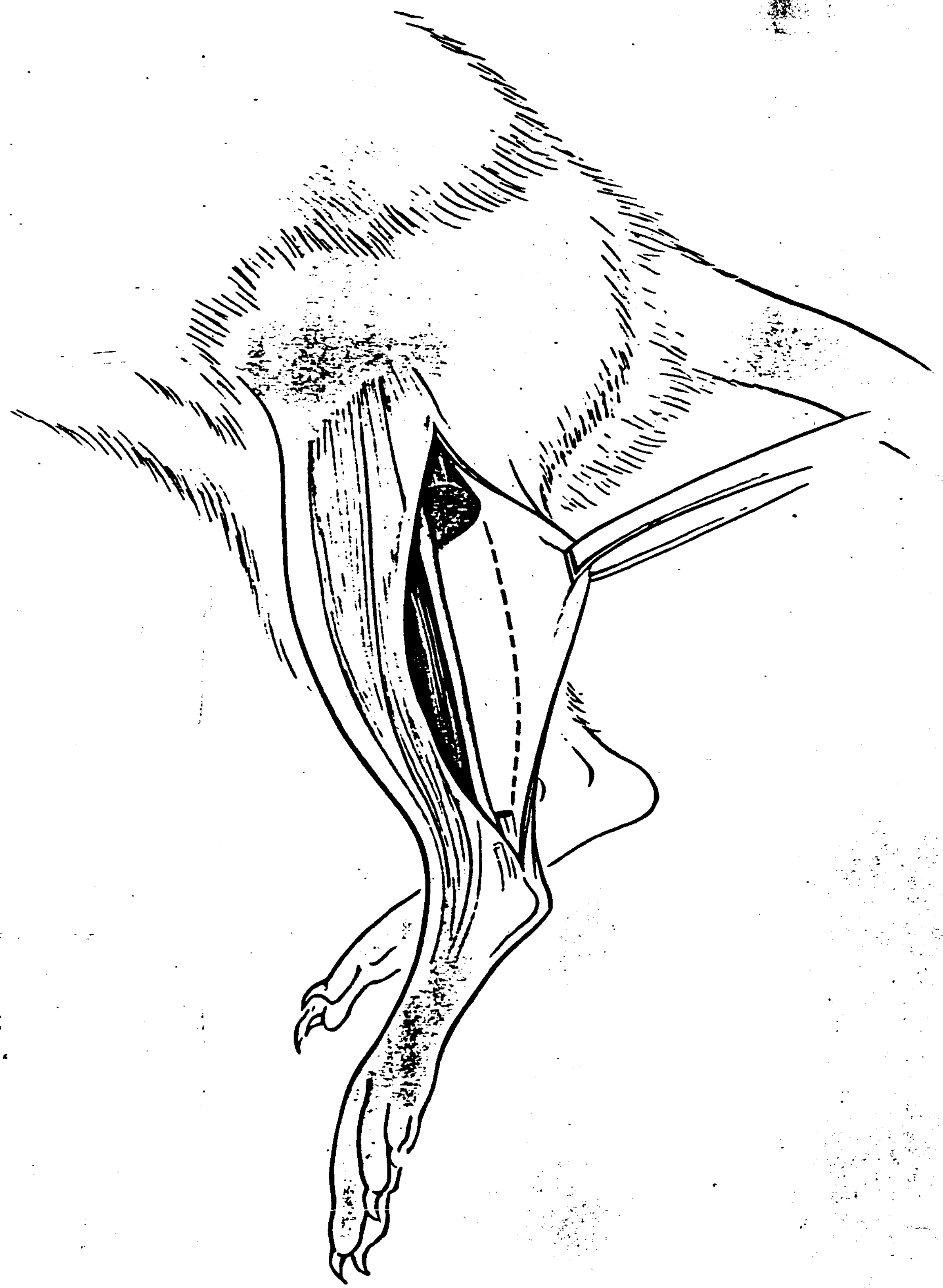
1. that the rabbit model is a good approach to study disuse osteoporosis in a non-growing (vs. rat) animal.

2. the skeletal effect has been confirmed in another larger species and, again, indicates the superiority of the single pulse in this particular biological system.

In both the rat and rabbit models there was consistency from the program in producing significant osteoporosis in control animals and in reducing the loss in treated animals.

FIGURE I Rb

Drawing of Operative Procedure
for Rabbit Disuse Osteoporosis Model



MECHANICAL TEST RESULTS FOR RABBIT OS CALCI

TABLE 1 Rb

Experiment Groups (Adults)	Animal	Limb Code	PEMF Exposed	Trabecular Load (in. lbs.)	Cortical Load* net load (lbs.)	Cortical Load* displacement (in inches)	Residual Energy ** (in. lbs.)
CONTROLS (Standard Laboratory Cage Living)	Y	(1)	—	3.07	61	.06	3.58
		(2)	—	2.52	56	.05	2.69
	Z	(1)	—	3.46	69	.08	6.69
		(2)	—	3.29	83	.08	3.83
RABBITS: CASTED/OPERATED CONDITIONS FOR 5 WEEKS:							
control limb	5B	L	—	2.85	41	.11	1.14
experimental limb		R	Pulse Burst	4.49	35	.06	4.78
control limb	5S	L	—	3.91	40	.04	4.44
experimental limb		R	Single Pulse	6.35	54	.06	4.32
RABBITS: CASTED/OPERATED CONDITIONS FOR 7 WEEKS:							
control limb	7B	R	—	2.73	33	.05	4.59
experimental limb		L	Pulse Burst	2.69	31	.07	5.15
control limb	7S	L	—	2.06	18	.07	4.18
experimental limb		R	Single Pulse	3.32	28	.06	5.12

* Load: represents radial splitting (yield point) of cortex
Displacement: excursion required to cause cortical splitting

** Residual Energy: Integrated area under the curve, determined from the point of maximum deformation to the end of excursion.

AUXILLARY STUDIES DEVELOPED FROM THIS PROJECT AT OTHER INSTITUTIONS

After the first sets of significant results of coil effects on the rat osteoporosis model were available, Dr. Emily Holton and Dr. Eric Sabelman at NASA-Ames in San Francisco expressed interest in using our coils and equipment in their studies of bone formation and destruction. In late 1977, Dr. Sabelman was provided with single coils to survey in his studies on osteogenesis in tissue culture. Histological data is being collected on modification of skeletal behavior in culture under the influence of the fields.

As a prelude for expanding the size of coils for whole body coverage in man, a preliminary safety and efficacy check was planned and started for nearly whole body exposure of restrained primates in collaboration with Dr. Don Young at NASA-Ames. Also, with so much emphasis in the past on calcium balance studies, it was determined to investigate whether the established technology would alter negative calcium balance in these primates, as a preliminary step to humans.

The initial pilot monkey studies of field effects on calcium balance and local bone loss were started in mid 1978 at NASA-Ames with equipment made available by Electro-Biology Inc. In these initial studies a long coil was used for the spine, round coils for the legs, and, unfortunately, at that time a pulse burst signal was chosen. These decisions were made prior to the data from the "100" Series in the rat model research was completed from which we learned that the round coils and pulse bursts are considerably less effective than the rectangular or square coils delivering a single repetitive pulse. In hindsight and in view of the low induced voltages and wrong pulse shapes, it would have been surprising if any progress had been made. In the preliminary report of results from spring of 1979 the data demonstrated no protective effect of PEMF on local osteoporosis in the tibia or on negative calcium balance. The project was felt to be a technical failure but should be repeated with improved technology.

In September of 1977 Dr. Richard Cruess, Professor and Chairman of the Department of Orthopedic Surgery at McGill University in Montreal, began to conduct a joint program to investigate the effects of the fields on biochemical parameters in this osteoporosis model. The studies used the "combined approach" rat model and the single pulse for his studies of both matrix and mineral turnover using labeled proline and Ca^{45}. Results of his work will be presented at the Orthopaedic Research Society Meeting to be held in February 1980. An extended abstract of the material presented at that time will be available in Orthopaedic Transactions published by the Journal of Bone and Joint Surgery.

Results to date from Dr. Cruess's study indicate an effect of the fields on calcium, hydroxyproline, proteoglycan and collagenase of rats subjected to the combined procedure (equivalent to our Group II controls), and a major modification by the single pulse of this pattern (in rats equivalent to our Group III animals). The following results were obtained: (1) the incorporation of C^{14} proline into hydroxyproline was significantly increased in PEMF exposed rats over controls; (2) incorporation of C^{14} glucose into hexosamine was significantly increased in rats exposed to PEMFs over control animals; (3) The uptake of Ca^{45} was more than doubled in treated rats vs. controls; and (4) There was nearly a ten fold decrease in collagenase activity in treated rats vs. controls.

In summary, the biochemical data from Dr. Cruess's endeavors inforce the results obtained during this contract period: namely, bone loss can be produced with the "combined approach" rat model and this loss can be largely prevented by exposing the animals to the effects of pulsing electromagnetic fields (single repetitive pulse). The biochemical results suggest an effect both on bone destruction (decrease) and formation (increase).

PRESENTATION OF RESULTS AT MEETINGS/PUBLICATIONS

During the contract period, members of the Orthopaedic Research Laboratories associated with this investigation of electromagnetic modification of disuse osteoporosis have presented selected results from this project at professional meetings and have prepared technical papers for publication.

In November of 1977, the Principal Investigator participated in a NASA sponsored conference in San Francisco at the U.S.P.H.S. Hospital to review preliminary results of impact (mechanical) loading as a means to modify bone loss associated with bed rest. Preliminary results of the rat model were presented.

In February of 1978, the Principal Investigator and the Senior Technician on this project, Lee S. Bassett, presented a paper at the 24th Annual Orthopaedic Research Society Meeting held in Dallas, Texas. An extended abstract entitled "An Improved Model for Producing Rapid Disuse Osteoporosis in the Rat Tibia" summarizes the information presented. This abstract appeared in the May 1978 volume of <u>Orthopaedic Transactions</u> published by the <u>Journal of Bone and Joint Surgery</u>. (See Appendix I)

The results of the studies of field effects on disuse osteoporosis were presented by the Principal Investigator in Philadelphia, October of 1978, at a conference organized by Dr. Carl T. Brighton to examine "Electrical and Magnetic Control of Musculoskeletal Growth and Repair". A paper presented at this time, entitled "Prevention of Disuse Osteoporosis in the Rat By Means of Pulsing Electromagnetic Fields" is to be part of a volume

Presentation of Results at Meetings/Publications, continued.

being published by Grune and Stratton. This collection of conference papers is currently in press and will be available in September of 1979 under the title: Electrical Properties of Bone and Cartilage: Their Clinical Application. (See Appendix II)

ACKNOWLEDGMENT

Mr. Jack Ryaby, President of Electro-Biology Inc., West Caldwell, New Jersey, for his assistance in making many small adjustments to adapt the leased electromagnetic equipment to proper operation in these studies.

Extended Abstract Entitled:

"AN IMPROVED MODEL FOR PRODUCING DISUSE OSTEOPOROSIS IN THE RAT TIBIA"

Published in: Orthopaedic Transactions, Journal of Bone and Joint Surgery May, 1978

L.S. Bassett, G. Tzitzikalakis and C.A.L. Bassett, M.D. Orthopaedic Research Laboratories, Columbia University, N.Y., N.Y. Since the manned space program began, increasing emphasis has been placed on combatting disuse osteoporosis. Many models have been developed to approximate the bone loss which occurs progressively, during weightlessness. These have included bed rest, bouyancy, nerve section, tenotomy, and plaster or other forms of skeletal immobilization. Previous reports of localized disuse osteoporosis, in rats indicated a preponderance of lumbar root section, tenotomy, or plaster immobilization to induce bone loss. Ash weights and breaking strengths of whole bones were the major techniques used to assay results and trabecular bone, the most "metabolically active" component, was not studied as an independent factor. In mature animals none of these methods achieved alterations in excess of 20% after 28 days of treatment. The present model was developed to study trabecular bone and to produce a rapid reduction in this component and to test ameliorative effects of pulsing electromagnetic fields. The results indicate that the major portion of trabecular bone in the proximal tibial metaphysis is lost within 28 days. Following excision of the gastrocnemius, soleus, and tendo Achilles bilaterally from 260 $\pm$ 10 gm female Sprague Dawley rats, both hind limbs were immobilized in a plaster spica, from upper thorax to the toes. The animals were further fixed by two transcutaneous Kirschner wires piercing two proximal tail vertebral bodies. These pins were, in turn, placed in two adjustable "C"-shaped plastic yokes which were coupled to a lucite bar extending caudally along the dorsum of the cast. A plaster pedestal attached to the ventral surface permitted the animal to be "suspended" in space and freed the forequarters for feeding. Two groups of animals were established; littermate free-ranging, controls, which were housed in cages and an experimental group which had been operated and casted. All animals were fed, ad libitum, and sacrificed after 28 days. After removal of soft tissues, fresh tibiae were x-rayed, with control and experimental pairs on the same film. A segment of the proximal tibia, measuring 0.4 inches in length was removed and mounted in dental acrylic with the open medullary cavity facing upward and the tibial axis aligned vertically. A .06 inch diameter cylindrical plunger, 0.4 inches long was fitted to a 100 lb. load cell head of an MTS (Materials Test Systems), Series 810. After centering the plunger directly over the transected tibial shaft, it was advanced into the medullary canal and metaphysis, for a distance of 0.25 inches at a rate of 0.625 inches/min. A load-deformation curve was produced for each specimen. Free-ranging, control animals, produced peak loads (at maximum plunger excursion) of 14.7 ± 3.8 lbs. (n=20). Experimental animals, which had been operated and casted, produced peak loads of 1.8 ± 1.3 lbs. (n=16). These differences are significant at $p>.001$ and represent reduction in trabecular loading capability in excess of 80% in 28 days. The mechanical testing results of control and experimental tibiae were compared with the radiographic and histologic appearance of longitudinal sections, taken through the center of the plunger track, and found to exhibit a high degree of correlation. Specimens with the lowest load value demonstrated a marked reduction in the number and thickness of metaphyseal trabeculae. This study demonstrates that it is possible to effect, quickly, a significant loss of cancellous bone mass in the proximal tibia by combining myectomy, tenotomy, and plaster immobilization. The model has proven its value in assessing the effects of time-varying electromagnetic fields with different pulse characteristics. In fact selection of the proper pulse parameters can prevent, completely, the bone loss which occurs in this model system. These studies were supported by NASA contract no. NAS 9-14931 and a grant from the Bioelectric Research Foundation.

PREVENTION OF DISUSE OSTEOPOROSIS IN THE RAT BY MEANS OF PULSING ELECTROMAGNETIC FIELDS

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Supported by NASA contract NAS-9-14931.

Introduction:

Osteoporosis is a condition characterized by a loss of bone mass[1]. It occurs as a result of a variety of causes, acting individually or synergistically. These include, among others, chronically restricted calcium intake; a chronically- altered dietary Ca: P ratio, skewed in favor of phosphate; hyperparathyroidism; hypersteroidism, whether endogenous or exogenous; genetic errors, such as osteogenesis imperfecta and hypophosphatasia and disuse, immobilization, or weightlessness. In fact, bone mass reflects the balance between formation and destruction. If, for example, osteogenesis is diminished, osteoclasis can remain normal, increase, or decrease by a lesser degree, and osteoporosis, eventually, will result. One of the keys in preventing a diminished bone mass, therefore, centers on maintenance of an adequate rate of bone formation.

Bone is similar to any product manufactured in a factory, in that it requires an appropriate supply of raw materials (eg., calcium, phosphate,amino acids, Vitamins C & D), functional machinery (e.g., mesenchymal cells and osteoblasts), and a source of regulated energy (e.g., mechanical electrical). If any of these three essential factors is inadequate or absent, the end product is limited or non-existent. This report focuses on the third of these factors, namely, regulated energy.

Ten years ago, Wolff's law was modified to state: "The form of a bone being given, the bone elements place or displace themselves in the direction of the functional pressure _and increase or decrease their mass to reflect the amount of pressure_. " Italics, here, emphasize the role that cyclic mechanical deformation plays in the maintenance of bone mass. The statement was included in a treatise on the biologic significance of piezoelectricity as part of a widening proposal that electrical phenomena, ultimately, regulate the activity of bone forming cells[2].

Certainly, there is ample evidence that bone mass is controlled by mechanical factors. A reduction of deforming forces by bedrest, immobilization, paralysis, bouyancy, or weightlessness, all[3,4,5,6], result in a decrease in skeletal volume (osteopenia). On the other hand, increased cyclic loading (particularly impactive) causes an accretion of osseous mass (e.g., hypergravics[7,8] and exercise, such as tennis[9]). The end result of repeated mechanical deformation and muscle contraction is an increase in the pulsing electrical currents in bone through piezoelectric and streaming phenomena[10,11,14]. If these electric currents are the source of the regulated energy which "runs" the "machinery" in a bone "factory", it should be possible to maintain or improve bone production, in the absence of mechanical input merely by inducing appropriate electrical currents in bone. In other words, it was hypothesized that a bone forming cell would not "ask" where the modification in its electrical environment came from, but, rather, what the modification "meant" to its functional state. This study demonstrates the feasibility of substituting electric currents induced by a pulsing electromagnetic field (PEMF) for those mechanically-produced and, thereby, maintaining skeletal mass. Furthermore, the pattern of cellular dynamics observed in these investigations adds credance to the hypothesis that Wolff's law is a manifestation of a bio-feedback system in which an electromechanical transducer (bone matrix) regulates, electrically, the activity of osseous elements[10,11,12,13,14].

Against this background, a major thrust of the experimental program was to develop a practical method for combatting the disuse osteoporosis and negative calcium balance associated with prolonged space flight[5]. Technologically, it made use of observations in animals and humans that time-varying electromagnetic fields could be inductively coupled to bone to produce biologically-significant electrical voltages and currents[15,16,17,18]. The method, which employs electromagnetic coils mounted outside the body, had already been demonstrated to hasten fracture healing and to trigger the repair of non-unions and pseudarthroses (described elsewhere in this volume). Since the electromagnetic fields appeared to be safe, could be scaled for local or whole body use, and were surgically non-invasive, they, clearly, provided an approach not feasible with electrode-delivered currents. The system, theoretically, could be of great importance to NASA for maintaining skeletal mass in astronauts[19]. It, also, might prove to be an essential adjunct in treating the more than 5,000,000 U.S. females above 65 years with clinically-significant osteoporosis[20] and who, because of pain, disability, poor reflexes, or motivation are unable to carry out effective impactive exercise programs.

Osteoporosis, in its earliest phases, characteristically, involves cancellous bone. Whether this pattern in spongy bone is a manifestation of a metabolically active state, where turnover is rapid, or a role as an energy-damping (absorbing) material, or both, is not clear. For practical purposes, however, this investigation has focused on the effects of disuse in metaphyseal bone. It has studied this region by radiographic, mechanical and histologic techniques to survey and define the ability of PEMFs to maintain bone mass under conditions in which the loss of cancellous bone is rapid[21].

METHODS

Adult, female, non-breeding 260 gm ± 10 gm Sprague Dawley rats (Camm Labs) were utilized in this study. Two strains of these animals were employed in different phases, one raised in a pathogen-free environment (A), one raised in a normal environment (B). The former (A) strain was characterized by a normal weight gain of 1-2 grams per week, while the latter (B) gained 8-10 grams per week. Free ranging control animals were allowed unlimited activity in rat cages and fed ad libitum on water and Purina rat chow. These animals were carried for 28 or 56 days before sacrifice and testing as noted below.

Two experimental groups of rats were established, one an operated/casted control and the other an operated/casted, PEMF-exposed. The operative procedure in both experimental groups (control and treated) was identical. It consisted of a bilateral removal, under chloral hydrate anesthesia, of the gastroenemius and soleus muscles, together with the tendo-Achilles, and a neurectomy of the posterior-tibial and peroneal nerves at the level of the popliteal space. Following skin closure the animals were casted in plaster from thorax to toes, taking care to pad bony prominences. A plastic bar was added dorsally to the cast and extended caudally over the tail. This holder served to mount two yokes through each of which a threated Kirschner wire transfixed adjacent vertebral bodies at the base of the tail. This arrangement prevented the rats from "wiggling" free from the spica as disuse robbed them of body weight. Each rat was "suspended" in air by a plastic dowel, mounted to a tray and fixed by plaster to the thoracic portion of the cast. Forelimbs were free to aid in feeding from a trough placed immediately in front of the rats. A gravity-fed water nipple permitted drinking, ad libitum.

Control animals were maintained in the same room as experimental animals under treatment, but at a distance of 15 feet from the coils. Electromagnetic fields in this portion of the room were monitored with a coil-probe and were found to be 6 orders of magnitude less than those used for treatment. Each PEMF-exposed animal was placed in between two vertically-mounted, Helmholtz-aiding, "O"-shaped coils, 24 hours post-operatively. For studying the preventative capability of the system, rats were exposed to the fields, 24 hours a day for 28 days, before sacrifice. In a preliminary study of the fields' ability to restore bone mass, animals were carried for 28 days as experimental controls (operated/casted) and then placed in their casts within the coils for an additional 28 days before study.

Two basic patterns of pulsing electromagnetic fields were employed. One consisted of a single, quasi-rectangular primary (positive-going) waveform, 325 usec wide, and inducing 1-1.5 mv/cm of bone. This pulse repeated at 65-72 Hz. The second pulse was characterized by a burst of 20 positive-going, quasi-rectangular pulses, 200 μsec wide, each separated by a negative-going pulse $\approx$30 usec wide. Bursts were repeated at 10 Hz, the pulse amplitude being adjusted to induce 1.0-1.5 mv/cm of bone. Equipment for this pulse was leased from EBI*.

After sacrifice with an overdose of barbiturate, the tibiae were dissected from each animal and groups of free-ranging control, experimental control and experimental-treated bones X-rayed on a single, industrial-grade plate. All specimens were maintained in a moist state with Tyrode saline during dissection, X-ray and preparation for final mechanical testing. After X-ray, 0.4 inches of the proximal tibiae were removed with the aid of calipers and a jeweler's saw. These specimens were mounted, vertically, in an aluminum cup by means of quick-setting dental acrylic, which encompassed the articular surface and the ossification center. The cup and specimen were placed in a set of vise-grip jaws, fixed to the piston of a series 810 MTS (Materials Testing System), so that the open medullary cavity faced upward, with the long-axis of the tibia vertically aligned. A 0.06 inch diameter stainless steel plunger was fitted to a 100 lb. load cell head. After centering the plunger directly over the transected tibial shaft, the piston was activated and the plunger advanced into the medullary canal and the metaphysis for a distance of 0.25 inches, stopping just short of the epiphyseal plate, at a rate of 0.625 inches/min. (Fig.1). A load/time curve was produced for each specimen by an X-Y plotter. Following mechanical tests, specimens were hemi-sectioned longitudinally,

*Electro-Biology, Inc., P.O.Box 682, W. Caldwell, N.J. 07006. Patented.

through the plunger track, and fixed in 10% buffered neutral formalin. After decalcification in E.D.T.A., tibial sections were embedded in paraffin, sectioned and stained with H & E or Masson Trichrome. Histologic evaluation, initially, was recorded by observers with no knowledge of specimen origin.

RESULTS

Generally, there was excellent concurrence between radiographic appearance of specimens, their mechanical test values, and the histologic evaluation. All animals in the two experimental groups of both A & B strains lost weight equally over the 28-day interval. The mean loss of experimental controls (operated/casted) Strain A and Strain B was 36 $\pm$ 10 grams and for the experimental, treated groups, 30 $\pm$ 18 grams. Free-ranging controls, as noted earlier, gained 1-2 grams/week for Strain A and 8-10 grams for Strain B. A typical free-ranging control mechanical result is seen in Fig. 2. Chart 1 compares the effects of casting and operation on tibial metaphyseal bone characteristics in the rapidly growing Strain B rats. On the left, the bar demonstrates a mean peak load of 14.8 $\pm$ 3.6 lbs. for free-roaming controls after 28 days. The second bar presents the loads for casted/operated experimental controls after a similar interval. The peak value in this group was 1.8 $\pm$ 1.2 lbs. and a typical trace is demonstrated in Fig. 3. The third and fourth bars summarize additional groups of Strain B animals for the sake of comparison. From these mechanical data, several observations are appropriate. First, there is a profound loss of mechanical integrity in the proximal tibial metaphysis of the rat, following 28 days of post-operative immobilization. This amounts to nearly 90% when compared to free-ranging controls. The loss of tibial mechanical values from the normal 260 gram starting-weight, animal is more than 80%. From the second and third bars (Chart 1), it is evident that the two-phased procedure, combining operation and casting, has an additive effect on bone loss. Five extremities tested from the experimental-treated groups of Strain B rats, using the single pulse yielded a mean peak value of 11.4 $\pm$ 2 lbs., were significantly higher than the untreated casted/operated controls (p=0.001). This value is as high as the control, 260 gram rats, but not quite as high as the values for the free-roaming controls, after 28 days. A representative curve is seen in Fig. 4.

The results of the Strain A series of animals, in which both the single pulse and pulse burst were applied, is presented in Chart II. A value of 9.8 $\pm$ 3.2 lbs. was obtained for the free-roaming controls after 28 days. With the single pulse, the peak load was 8.3 $\pm$ 1.4 lbs., clearly, outside the range of the untreated controls, but, again, not quite equal to the maximal loads for free-ranging controls after 28 days. No significant protective effect was evident in the few animals treated with the pulse burst.

As noted earlier in the section on results, there was good concurrence between histological findings and mechanical results. Specimens producing low mechanical load values generally demonstrated a massive loss of trabecular bone in the metaphysis. In fact, the usual bollus of fractured trabeculae at the end of the plunger track, just short of the epiphysis, which was present in the mechanically-sound specimens, was not found in the osteoporotic tibiae (Fig. 5,6). The few residual trabeculae in these latter specimens were found at the periphery of the metaphysis, supporting the flaring "cortex" of the metaphyseal region. A decreased bone mass, also, was noted in the trabeculae of the ossification center. In the casted/operated controls, which produced the higher load values in the StrainArats, the major loss of trabecular bone was detected in the region nearest the diaphysis and it appeared, in these animals, that the marrow space (medullary canal) was "advancing" on the metaphysis. Mechanical test curves in such specimens were much flatter in the early phases of plunger advance than in normals. A significantly acute angle of load/slope might be reached only after one half to two thirds of the total plunger excursion. Integration of the area "under the curve" (energy absorbed) in these animals was significantly lower than normal.

Operated/casted animals exposed to a single pulse for 28 days demonstrated a much more normal histologic appearance in both the metaphysis and ossification center. In the vast majority, the usual bollus of fractured trabeculae was observed at the end of the plunger track and broad trabeculae occupied the sub-epiphyseal zone (Fig.7) In most of these animals, however, some decrease in trabecular bone toward the diaphysis was noted. This picture correlated well with the mechanical curves which had, uniformly, a decreased slope in the early phases of plunger entry, although peak loads may have reached, routinely, the range of normal-free-roaming 28 day controls.

In preliminary studies of the use of this modality to "cure" osteoporosis, the results have been highly variable, depending upon the field pattern in use. For example, it has been proven possible to improve the usual mechanical and histologic data produced by 28 days of immobilization, following operation, despite the continuation of immobilization for the additional 28 days of treatment in the coils. A total of 9 specimens out of 12 have demonstrated mechanical values after 56 days significantly above immobilized control values, with the maximum peak load slightly above 9 lbs. (Fig.8). The histologic picture approximates a normal metaphyseal bone mass (Fig.9). The nine successes occurred with pulses demonstrating slightly different characteristics than those used in the preventative program or in the other three animals in the "curative" program.

DISCUSSION

These results demonstrate that a mechanical test which assesses, primarily, the quality of cancellous bone can provide ample documentation of rapid loss of metaphyseal bone during disuse. These data, which are reported as peak load values reveal differences between normal free-roaming animals and control casted/operated animals of 90% after 28 days. They are highly significant. Even larger differences could be demonstrated if energy absorbed (area under the curve), instead of peak loads were reported. Early mechanical testing, as generally has been the case in previous studies[22,23,24,25,26], made use of whole bone and, although there were differences between groups, the changes were small compared to the alterations detected by the present method. In fact, it seems likely that the cancellous changes were being masked by the relatively lesser changes in cortical bone, in these early studies. It is suggested, therefore, that future investigations may be aided by this mechanical testing technique.

Since electrical currents were proposed to play a role in bone remodeling[10,12], the possibility of substituting electrical energy for mechanical energy to maintain skeletal mass has shaped some experiments involving osteoporosis models. For example, McElhanney et al reported in 1968[27] the use of capacitively-coupled, constant and dynamic "electrostatic" fields to modify disuse osteoporosis. These fields were found to be partially effective, an observation which has been confirmed recently by Martin[28]. Capacitative-coupling, however, requires an impractical "step-up" to massive voltages, if the inter-plate distances are increased from rat to human dimensions. Although bone atrophy has been approached with implanted electrodes with partial success in animals[29], it is highly unlikely that individuals at risk of developing osteoporosis could or would tolerate the multiplicity of electrodes required to treat all affected parts of the skeleton. Both of these electrical approaches, however impractical, help to establish the principle that electric energy can be substituted for mechanical energy to modify bone mass. PEMF's have a practical advantage over these other approaches and, furthermore, appear to be much more effective in producing results which may, ultimately, have clinical significance.

This investigation, as often is the case, raises more questions than it answers. For example, the 260 gram rat has an open epiphysis. Although growth is slow, it still is present. What roles, therefore, do growth and/or remodeling play in the observations reported herein? A partial answer to this question may be inherent in the differences in the amount of bone lost in the slow-growing (1-2 grams body weight increase/week), Strain A rats and the faster growing (8-10 gram/week) Strain B rats. It could be argued that the Strain A's have a slower rate of remodeling associated with their slower rate of body growth and this factor may have been repsonsible for the higher mechanical test values for the casted/operated controls, of this group. A more complete answer on this point may be forthcoming from a study, currently in progress, of immobilized and treated immature and mature rabbits. Interestingly, multi-nucleated osteoclasts were not observed in any of the rats. This observation which, also, has been made in other laboratories[30], obviously, does not imply that resorption was not present; it had to be in order to reduce cancellous bone strength by 90% in 28 days. Is it possible, therefore, that the protective effect of the PEMF's is to reduce resorption, or is the effect on increased turnover with the emphasis on accretion? Answers to these queries may be forthcoming from multiple flurochrome labels in both rats and rabbits.

Finally, it is clear from these and other studies[17,31] that bone mass can be altered beneficially by exposure to PEMF's. On this basis, a clinical investigation has begun to study the use of this technique to restore bone in individuals with osteolysis from metastatic cancer. Preliminary data indicate that pain from micro-fractures or impending stress/pathologic fractures can be controlled and bone mass restored in patients with this disease (Figs.10,11,12,13). Whether the modality will ever be put to practical use in space to prevent the negative calcium balance and osteoporosis of weightlessness must await further work. It is not inconceivable, however, that some day the treatment of senile and post-menopausal osteoporosis may involve the use of coil beds, along with correction of dietary and hormonal factors.

SUMMARY:

1. The loss of trabecular bone from the proximal tibial rat metaphysis, following casting, myectomy, tenectomy and neurectomy, has been documented radiographically, mechanically and histologically.

2. A new method of mechanical testing has proven to be a valuable and sensitive adjunct in detecting the extent of disuse osteoporosis in trabecular bone.

3. Treatment of casted/operated rats for 28 days with highly specific pulsing electromagnetic fields (PEMF's) prevents disuse bone loss to a significant degree.

4. This surgically non-invasive method, ultimately, may have practical application in controlling bone loss in astronauts and in patients with local or generalized regions of bone resorption.

5. Apparently, electrical currents, induced exogenously by PEMF's (Pulsing Electromagnetic Fields), can be substituted for endogenous electrical currents, generated piezoelectrically by cyclic mechanical deformation, to control the cells which determine bone mass.

6. These results buttress the hypothesis that Wolff's law is a manifestation of an electrically-controlled, bio-feed-back system.

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LEGENDS

Figure 1	Diagram of mounting for proximal tibial specimens in M.T.S. during mechanical testing.
Figure 2	X-Y plot of Time (x-ordinate) vs load (y-ordinate) for a normal free roaming Strain B (260 gram starting weight) after 28 days. Peak load at end of plunger excursion 15.6 lbs.
Figure 3	X-Y plot, as in Figure 2, but from a casted/operated (260 gram starting weight) Strain B after 28 days. Peak load 1 lb. - compare with normal control in Figure 2.
Figure 4	X-Y plot, as in Figure 2, but from a casted/operated, Strain B (starting weight, 260 grams) after coil treatment for 28 days (starting at 1 day post casting). Note peak load equals free roaming control (Figure 2) but that the initial slope is less than in Figure 2.
Figure 5	Longitudinal section, H & E, X 4 of same animal as depicted in Figure 2 (free roaming). Note plunger track (arrow) and bollus of fractured trabeculae at its bottom.
Figure 6	Longitudinal section, H & E, X 4 of animal similar to that depicted in Figure 3 (operated/casted control). Note almost complete replacement of trabecular bone by marrow. Plunger track not seen - no bollus of fractured trabecular material is present.

Figure 7

Longitudinal section, H & E, X 4 of animal similar to that depicted in Fig. 4 (operated/casted/treated). Note heavy metaphyseal trabeculae, clear by demarkated plunger track and mass of fractured trabeculae at its end.

Figure 8

Top trace - normal, free-ranging control after 28 days, peak load 12.6 lbs. Middle trace - operated/casted control after 28 days, peak load 1.5 lbs. Bottom trace - operated/casted control for 28 days and then placed in electromagnetic coils, still in cast, for an additional 28 days, peak load 9 lbs. with a more abrupt rise in load toward end of plunger excursion than the top trace.

Figure 9

Longitudinal section, H & E, X 4 of the animal depicted in Fig. 8, bottom trace. Compare with Fig. 3 and note, despite 56 days total immobilization (the last 28 of which were in coils), there are good metaphyseal trabeculae, a clearly defined plunger track and a bollus of fractured trabecular at its end.

Figure 10

B.M., a 53 yr. female, 3 years after radical mastectomy with skeletal metastases. A.P. radiograph of rt. hip and pelvis. Note lytic lesions in ischium and at level of lesser trochanter.

Figure 11

Same as Fig. 10, 1 month after application of electromagnetic coils. Note restoration of radio-density to lytic regions. Patient pain free.

Figure 12	Same as Fig. 10, but lateral radiograph of lumbar spine, demonstrating a large lytic lesion of the body of L_3.
Figure 13	Same as Fig. 12, but 1 month after institution of electromagnetic coil treatment. Lytic area appears to have filled with radio-dense material.

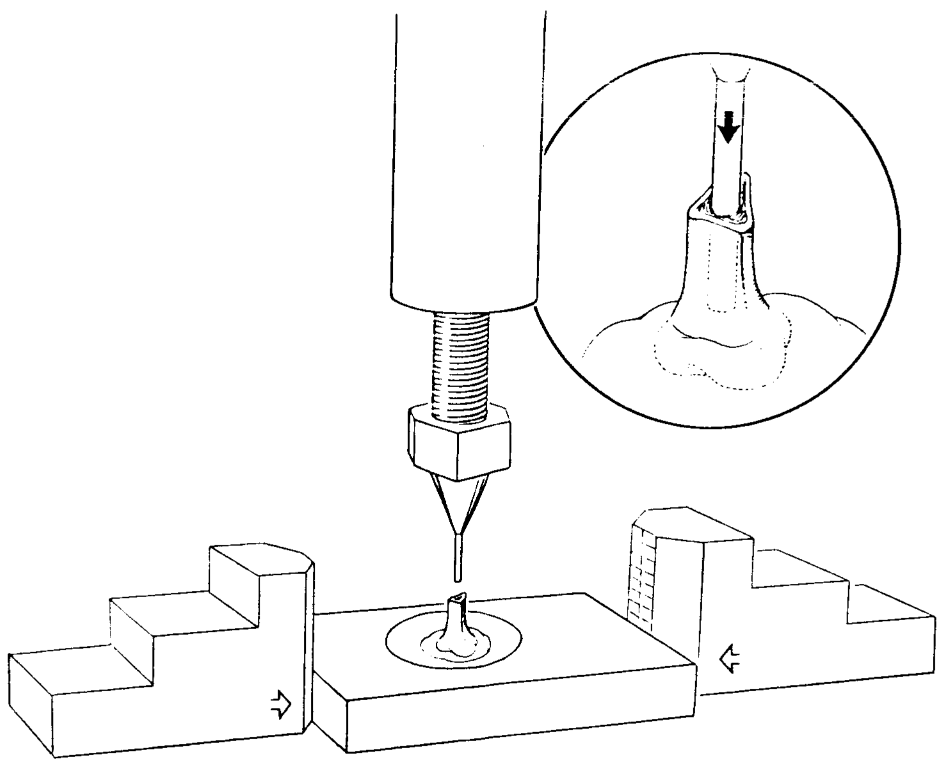
Chart I

Mean peak loads for proximal tibial metaphysis (with standard deviations) for Strain B Sprague-Dawley rats with a starting weight of 260 grams. Note increased efficiency of the model incorporating legs in plaster for producing a greater loss of trabecular bone ($p < .05$) than model with legs free. First three bars apply to animals maintained for 28 days under conditions noted at bottom.

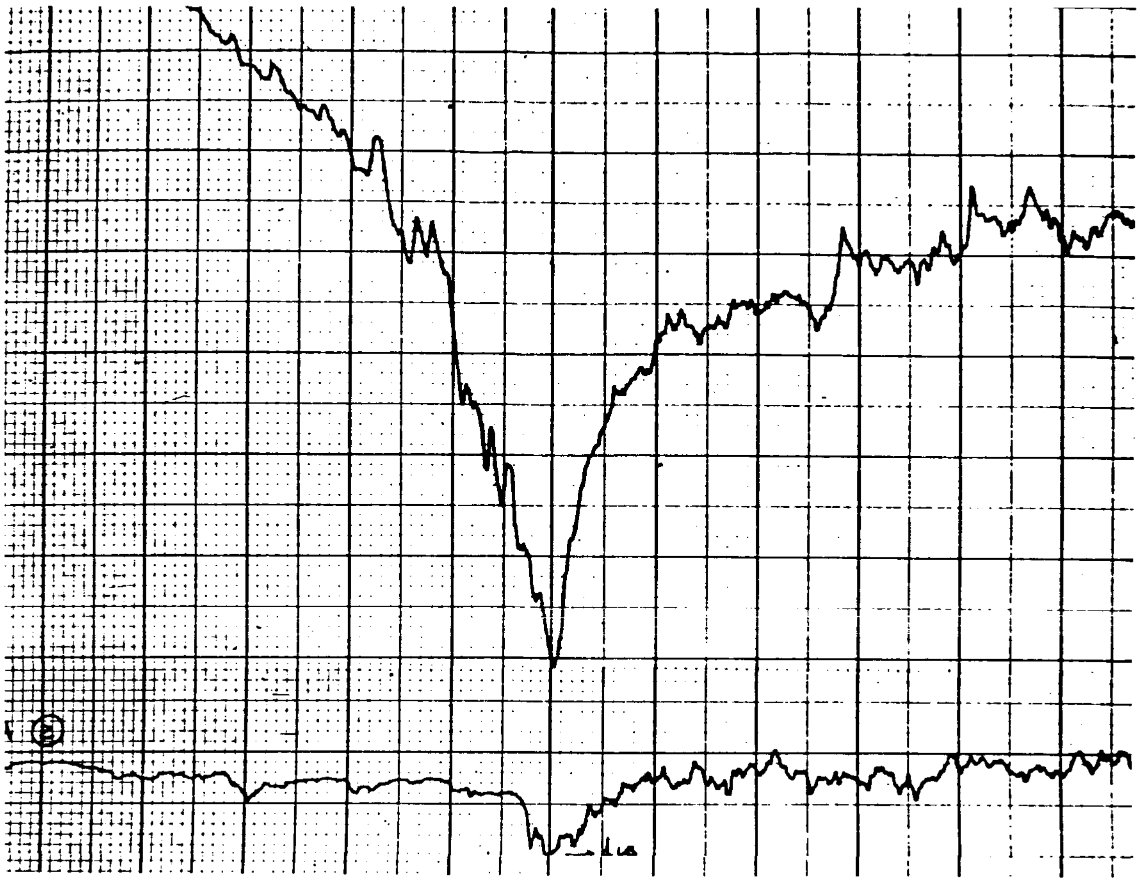
Chart II

28 day, mean peak loads (proximal tibial metaphysis) with standard deviations for Strain A Sprague-Dawley rats, starting weight 260 grams. The values for the free roaming and single pulse animals are significantly different than controls, ($P < .01$) but not from each other.

Figure 1.



Figures 2, 3, and 4.



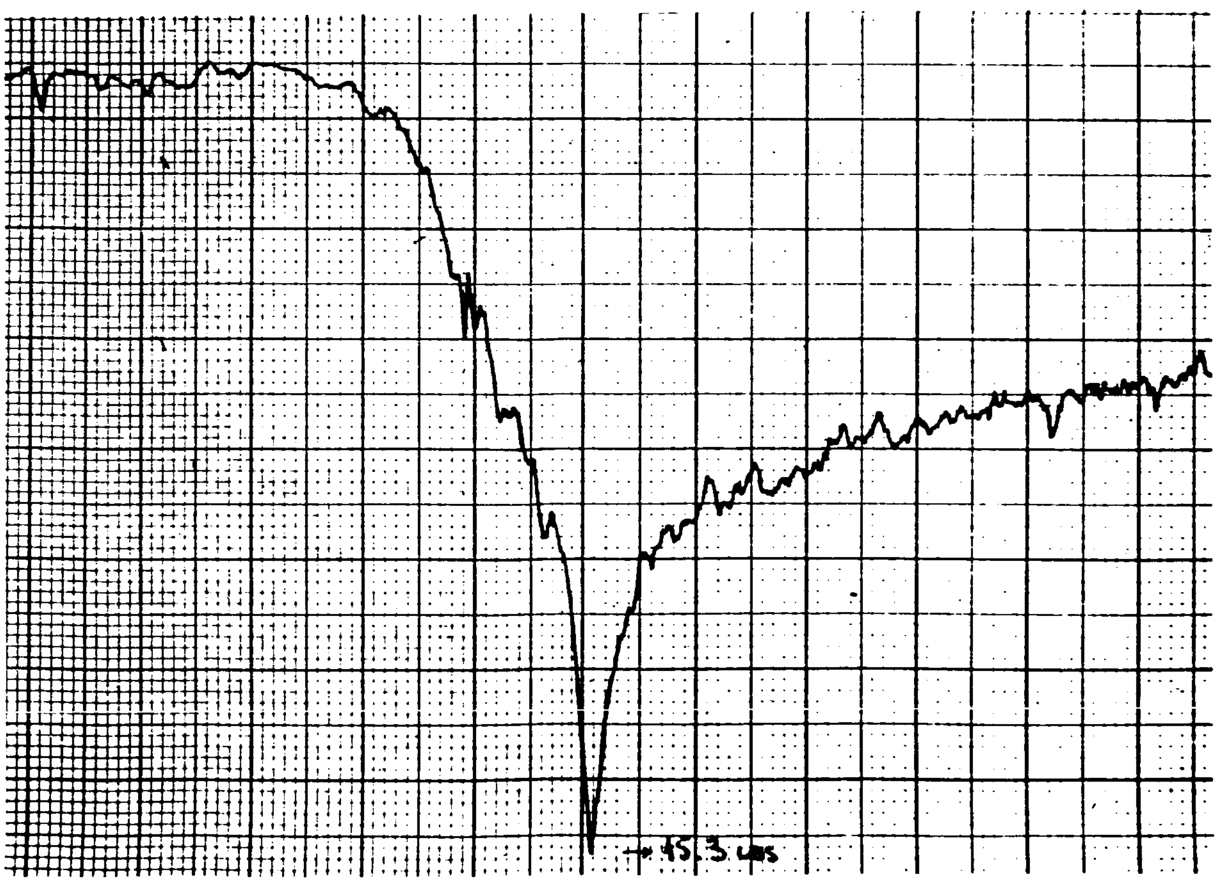


Figure 5.

Figure 6.

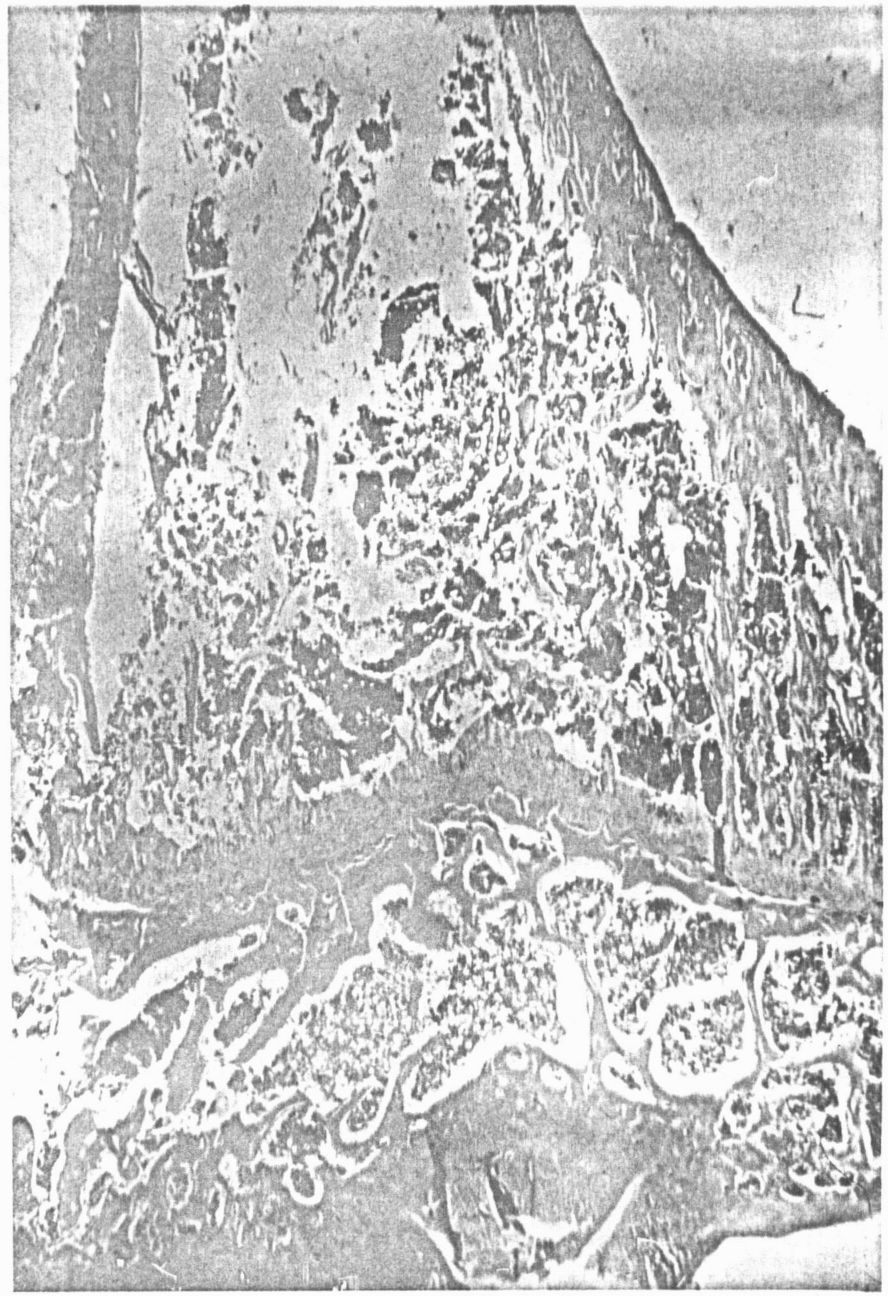
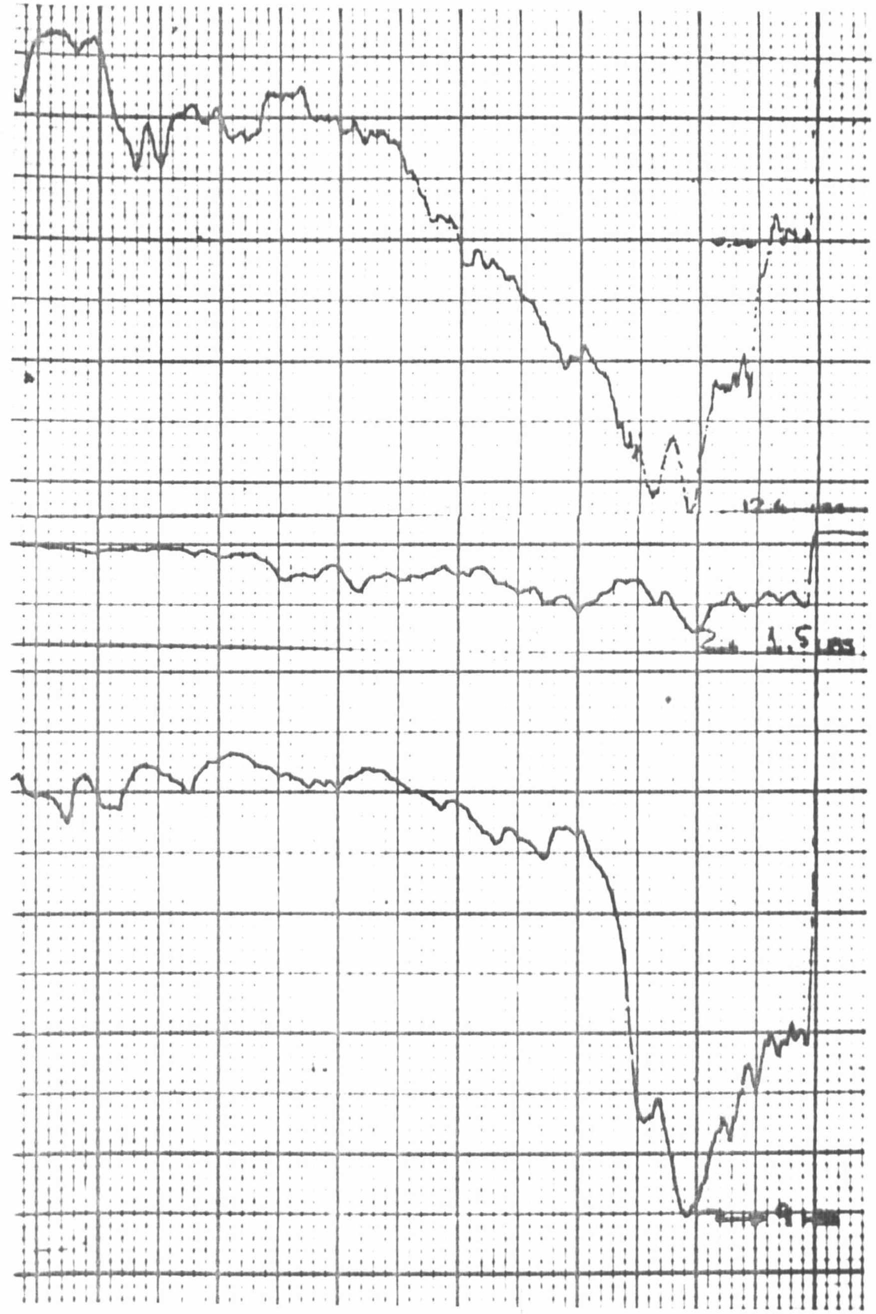


Figure 7.

Figure 8.



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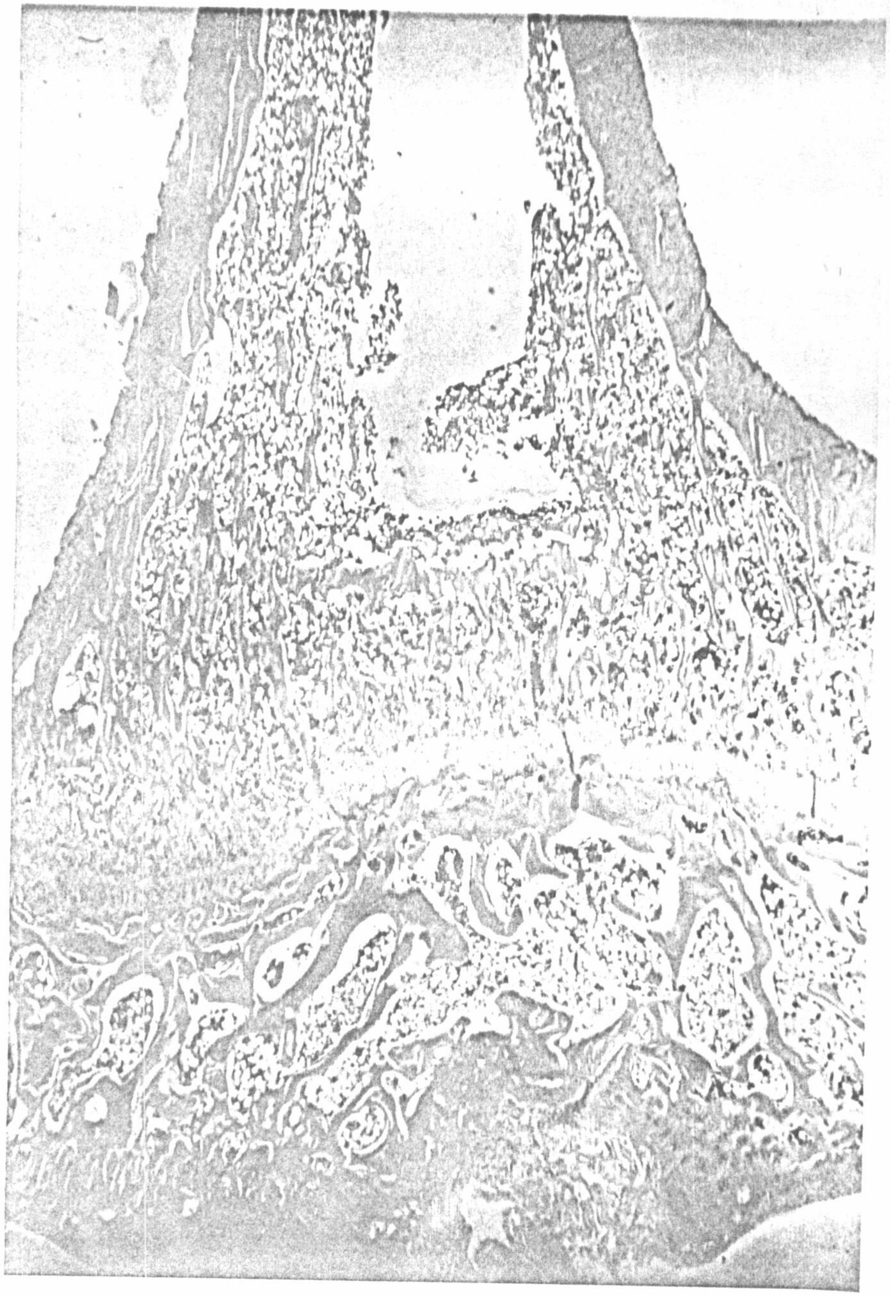

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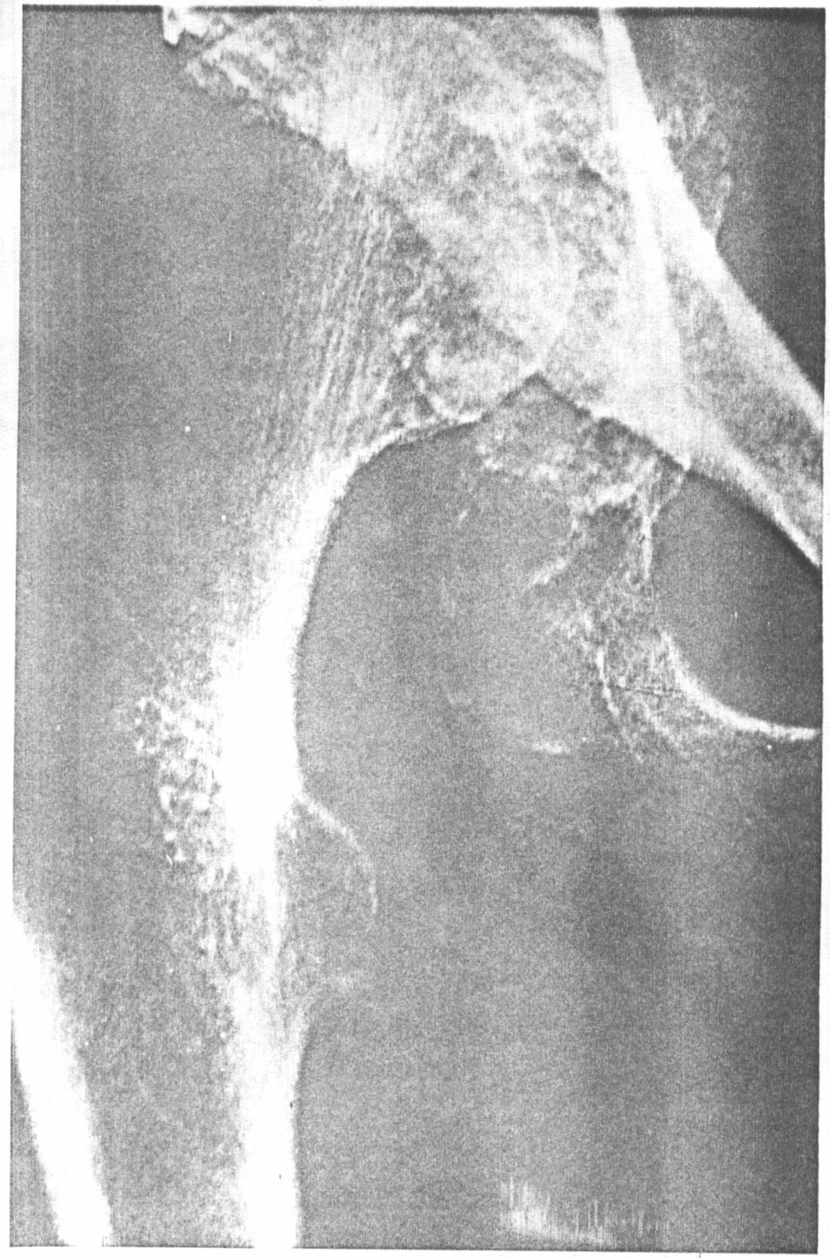

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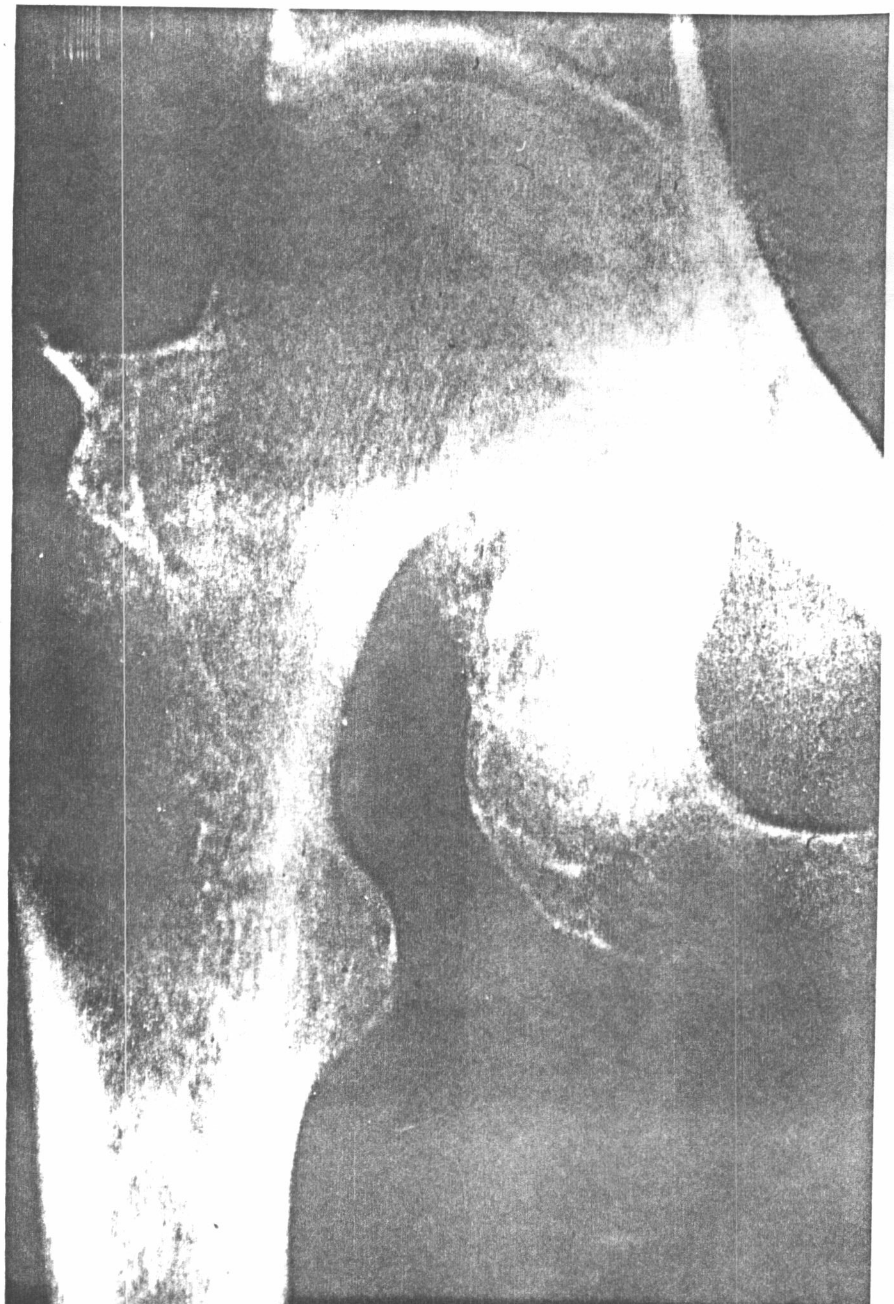

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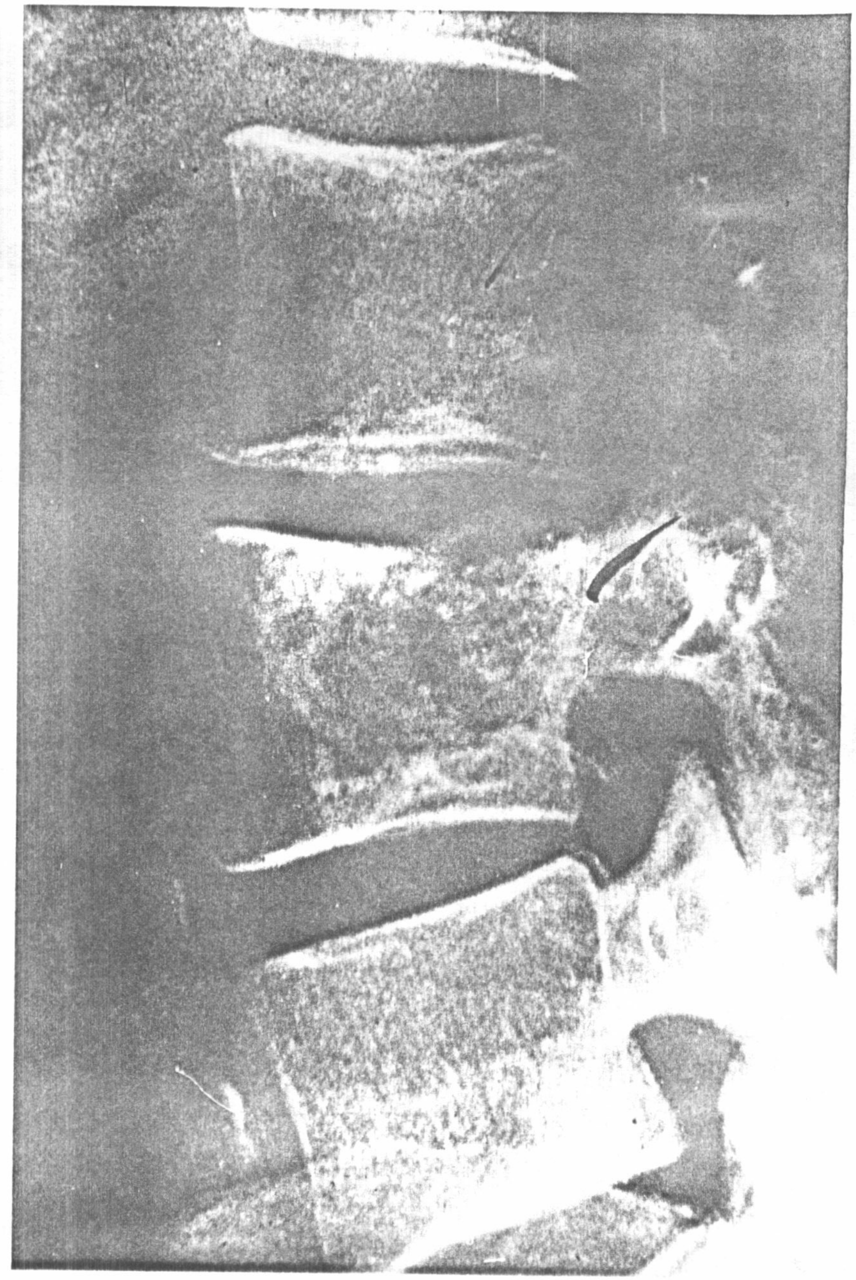


Figure 13.

Chart 1.

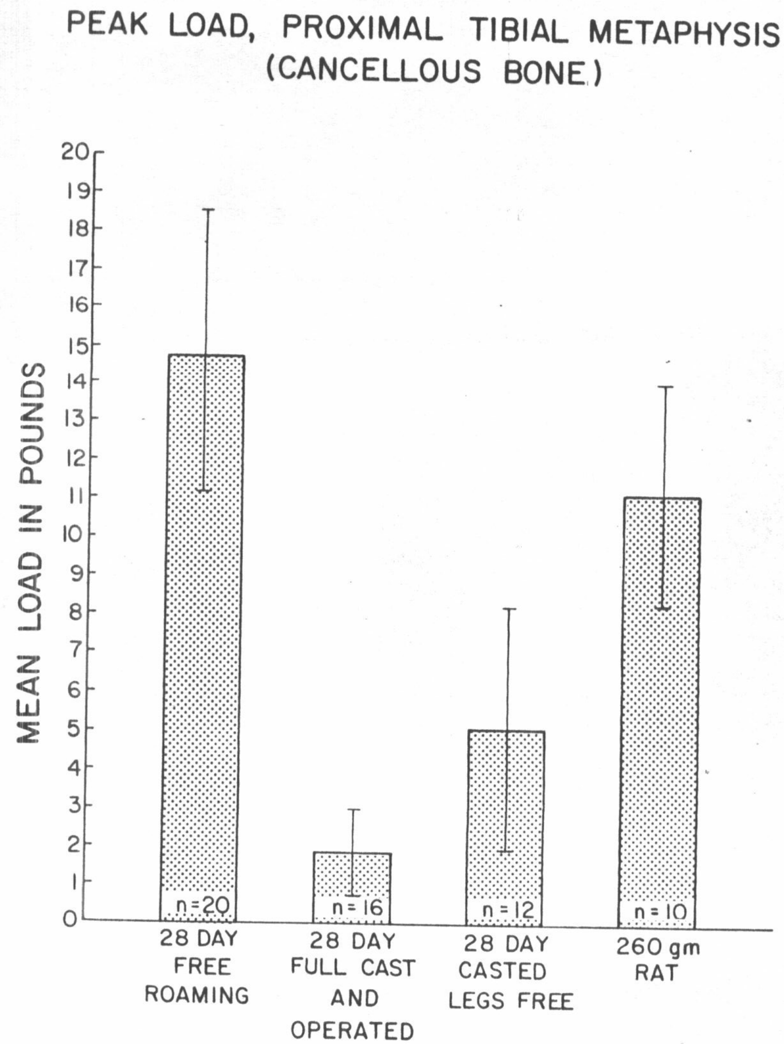
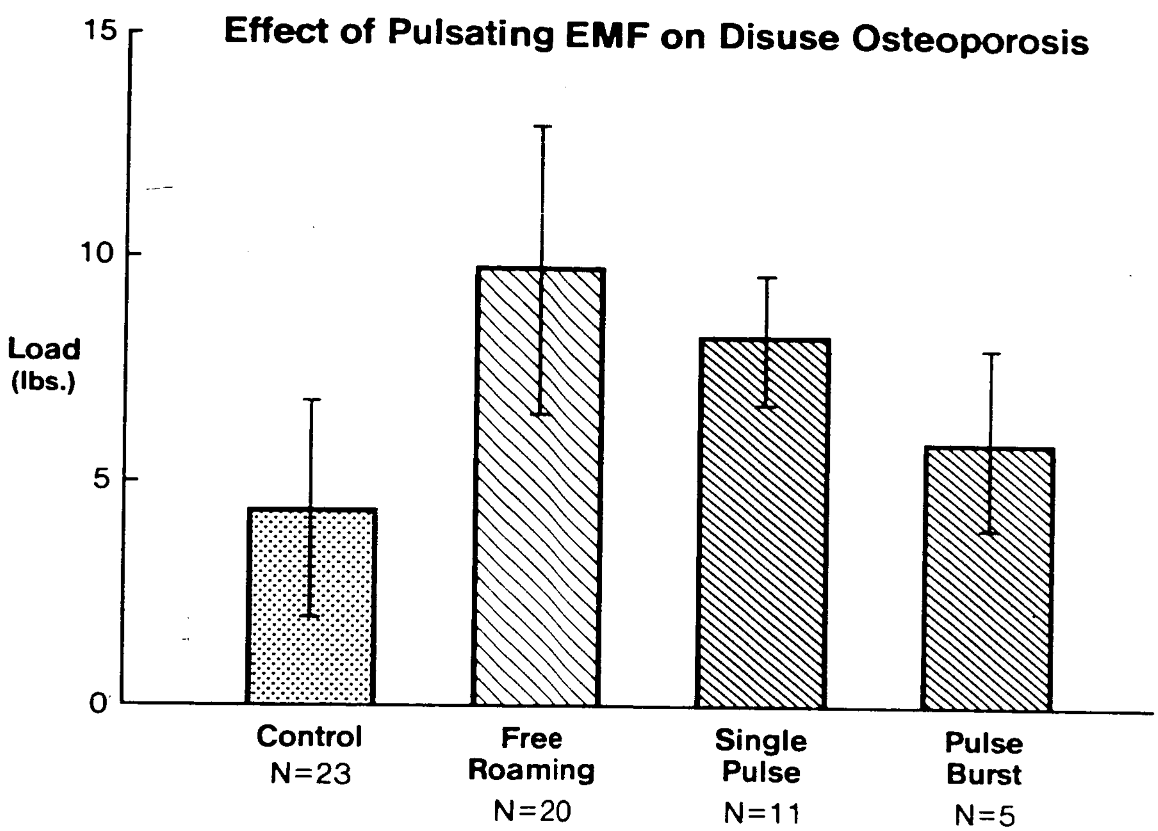


Chart II.


Effect of Pulsating EMF on Disuse Osteoporosis
15
10
5
0
Load
(lbs.)
Control
N=23
Free
Roaming
N=20
Single
Pulse
N=11
Pulse
Burst
N=5